

# **Total Maximum Daily Load Grant Program**

## ***Modeling Support and Bacterial Source Tracking For Big Cypress Creek Bacteria Assessment***

***TSSWCB Project 09-55  
Revision No. 1***

### **Quality Assurance Project Plan**

#### **Texas State Soil and Water Conservation Board**

prepared by

Texas AgriLife Research  
Texas Water Resources Institute,  
the  
Texas A&M University Dept. of Biological and Agricultural Engineering,  
and  
Texas A&M University Dept. of Soil and Crop Sciences

Effective Period: Upon Approval thru May 2011

Questions concerning this quality assurance project plan should be directed to:

Lucas Gregory  
Quality Assurance Officer  
Texas AgriLife Research, Texas Water Resources Institute  
2118 TAMU  
College Station, TX 77843-2118  
979.845.7869  
*lfgregory@ag.tamu.edu*

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## **Section A1 Approval Sheet**

Quality Assurance Project Plan (QAPP) for Project # 09-55, *Modeling Support and Bacterial Source Tracking for Big Cypress Creek Bacteria Assessment*.

### **Texas State Soil and Water Conservation Board (TSSWCB)**

Name: Mitch Conine  
Title: TSSWCB Project Manager

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

Name: Donna Long  
Title: TSSWCB Quality Assurance Officer

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

### **Texas AgriLife Research- Texas Water Resources Institute (TWRI)**

Name: Bill Harris  
Title: Acting Director, TWRI Project Lead

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

Name: Lucas Gregory  
Title: TWRI Quality Assurance Officer (QAO)

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

### **Texas AgriLife Research — Department of Biological and Agricultural Engineering (BAEN)**

Name: R. Karthikeyan  
Title: BAEN Assistant Professor, Project Co-Lead

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

**Texas AgriLife Research - Department of Soil and Crop Sciences (SCSC)**

Name: Terry Gentry

Title: SCSC Assistant Professor of Soil & Aquatic Microbiology; Project Co-Lead;  
Soil and Aquatic Microbiology Lab (SAML) Director

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

**North East Texas Municipal Water District (NETMWD)**

Name: Walt Sears, Jr.

Title: NETMWD General Manager

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

Name: Lee Thomas

Title: NETMWD Project Manager

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

**Ana-Lab Corporation**

Name: Bill Peery

Title: Ana-Lab Executive Vice President

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

Name: Roy White

Title: Ana-Lab Quality Assurance Officer

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

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## List of Acronyms and Abbreviations

BAEN	Department of Biological and Agricultural Engineering
BLSC	bacteria source load calculator
BST	bacterial source tracking
CAFO	confined animal feeding operation
CAR	corrective action report
COC	chain of custody
CBMS	computer based mapping system
CWA	Clean Water Act
DNA	deoxyribonucleic acid
DEM	digital elevation model
DQO	data quality objectives
EPA	United States Environmental Protection Agency
ERIC-PCR	enterobacterial repetitive intergenic consensus PCR
ERIC-RP	ERIC-PCR / RiboPrinting combination method
GIS	geographic information system
LDC	load duration curve
mRNA	messenger ribonucleic acid
MUG	methylumbelliferyl- $\beta$ -D-glucuronide
MS4	municipal separate storm sewer system
NA-MUG	nutrient agar with MUG
NELAC	National Environmental Laboratory Accreditation Conference
NETMWD	Northeast Texas Municipal Water District
NIST	National Institute of Standards and Technology
NLCD	national land cover data set
PCR	polymerase chain reaction
QA	quality assurance
QAPP	quality assurance project plan
QAO	Quality Assurance Officer
QC	quality control
QPR	quarterly progress report
RP	RiboPrinting
RPD	relative percent difference
rRNA	ribosomal ribonucleic acid
SAML	Soil and Aquatic Microbiology Laboratory
SCSC	Department of Soil and Crop Sciences
SELECT	Spatially Explicit Load Enrichment Calculation Tool
SM	Standard Methods for Examination of Water and Wastewater, 21 <sup>st</sup> edition
SOP	standard operating procedures
SSL	Spatial Sciences Laboratory
SSURGO	soil survey geographic
SWQM	surface water quality monitoring
tRNA	transfer ribonucleic acid
TAMU	Texas A&M University; College Station Campus
TCEQ	Texas Commission on Environmental Quality
TMDL	total maximum daily load
TPDES	Texas pollution discharge elimination system
TSSWCB	Texas State Soil and Water Conservation Board
TWRI	Texas Water Resources Institute
USGS	United States Geological Survey
UV	ultraviolet light
WPP	watershed protection plan
WQMP	water quality management plan
WWTF	wastewater treatment facility

### **Section A3: Distribution List**

Organizations, and individuals within, which will receive copies of the approved QAPP and any subsequent revisions include:

Texas State Soil and Water Conservation Board (TSSWCB)  
P.O. Box 658, Temple, TX 76503

Name: Mitch Conine  
Title: TSSWCB Project Manager  
  
Name: Donna Long  
Title: TSSWCB Quality Assurance Officer

Texas AgriLife Research, Texas Water Resources Institute (TWRI)  
2118 TAMU, College Station, TX 77843-2118

Name: Bill Harris  
Title: TWRI Acting Director, Project Lead  
  
Name: Lucas Gregory  
Title: TWRI Quality Assurance Officer

Texas AgriLife Research — Department of Biological and Agricultural Engineering (BAEN)  
2117 TAMU, College Station, TX 77843-2117

Name: R. Karthikeyan  
Title: Assistant Professor; Project Co-Leader

Texas AgriLife Research — Department of Soil and Crop Sciences (SCSC)  
2474 TAMU, College Station, TX 77843-2474

Name: Terry Gentry  
Title: Assistant Professor; Project Co-Leader; SAML Director

Northeast Texas Municipal Water District (NETMWD)  
P.O. Box 955, Hughes Springs, TX 75656

Name: Walt Sears, Jr.  
Title: General Manager

Name: Lee Thomas  
Title: Project Manager

Ana-Lab Corporation  
P.O. Box 9000, Kilgore, TX 75663

Name: Bill Peery  
Title: Ana-Lab Executive Vice-President

Name: Roy White  
Title: Ana-Lab Quality Assurance Officer

## **Section A4: Project/Task Organization**

The following is a list of individuals and organizations participating in the project with their specific roles and responsibilities:

**TSSWCB** –Texas State Soil and Water Conservation Board, Temple, Texas. Provides project overview at the State level.

### Mitch Conine, TSSWCB Project Manager

Maintains a thorough knowledge of work activities, commitments, deliverables, and time frames associated with the project. Develops lines of communication and working relationships between, TWRI, TAMU-BAEN, TAMU-SCSC and TSSWCB. Tracks deliverables to ensure that the tasks are completed as specified in the contract. Responsible for ensuring that the project deliverables are submitted on time and are of acceptable quality and quantity to achieve project objectives. Participates in the development, approval, implementation, and maintenance of the QAPP. Assists the TSSWCB QAO in technical review of the QAPP. Responsible for verifying that the QAPP is followed by TWRI, TAMU-BAEN and TAMU-SCSC. Notifies the TSSWCB QAO of particular circumstances that may adversely affect the quality of outputs derived from the model. Enforces corrective action.

### Donna Long; TSSWCB Quality Assurance Officer

Reviews and approves QAPP and any amendments or revisions and ensures distribution of approved/revised QAPPs to TSSWCB participants. Responsible for verifying that the QAPP is followed by project participants. Monitors implementation of corrective actions. Coordinates or conducts audits of field and laboratory systems and procedures. Determines that the project meets the requirements for planning, quality assessment (QA), quality control (QC), and reporting under the TSSWCB Total Maximum Daily Load Program.

**TWRI** - Texas Water Resources Institute (TWRI), College Station, Texas. Responsible for development of data quality objectives (DQOs) and a QAPP.

### Bill Harris, Project Lead

The TWRI Project Lead is responsible for ensuring that tasks and other requirements in the contract are executed on time and with the QA/QC requirements in the system as defined by the contract and in the project QAPP; assessing the quality of subcontractor/participant work; and submitting accurate and timely deliverables to the TSSWCB Project Manager.

### Lucas Gregory, Quality Assurance Officer

Responsible for determining that the QAPP meets the requirements for planning, quality control, and quality assessment. Conducts audits of field and laboratory

systems and procedures. Responsible for maintaining the official, approved QAPP, as well as conducting Quality Assurance audits in conjunction with TSSWCB personnel.

T. Allen Berthold, Project Manager

Responsible for ensuring the timely completion of project deliverables, fiscal oversight and project reporting.

**BAEN** – Department of Biological and Agricultural Engineering, Texas A&M University, College Station, Texas. Responsible for modeling activities associated with the Spatially Explicit Load Enrichment Calibration Tool (SELECT) and Load Duration Curve (LDC) development.

R. Karthikeyan, Assistant Professor, BAEN Co-Lead

Responsible for performing LDC analysis and SELECT modeling. Responsible for assisting in the development of a GIS inventory of the selected project watersheds and designing the watershed source survey. This includes ensuring that personnel involved in qualitative data assessment are adequately trained and a thorough knowledge of the QAPP and all SOPs specific to the analysis or tasks performed. Responsible for modeling oversight and ensuring that all QA/QC requirements are met, documentation related to the analysis is complete and adequately maintained, and that results are reported accurately. Responsible for ensuring that corrective actions are implemented, documented, reported and verified.

**SCSC** – Department of Soil and Crop Sciences, Texas A&M University, College Station, Texas. Responsible for conducting bacterial source tracking (BST) analysis.

Terry Gentry, Assistant Professor, SCSC Co-Lead; SAML Director

Responsible for performing BST analysis and related activities. This includes ensuring that laboratory personnel involved in generating analytical data have adequate training and thorough knowledge of the QAPP and all SOPs specific to the analyses or task performed. Responsible for oversight of all laboratory operations ensuring that all QA/QC requirements are met, documentation related to the analysis is complete and adequately maintained, and that results are reported accurately. Responsible for ensuring that corrective actions are implemented, documented, reported and verified. Monitors implementation of the measures within the laboratory to ensure complete compliance with project DQOs in the QAPP. Conducts in-house audits to ensure compliance with written SOPs and identify potential problems.

**NETMWD** – Northeast Texas Municipal Water District. Responsible for collecting field data and delivering QA data sets to BAEN and SCSC.

Walt Sears, Executive Director, NETMWD

Responsible for overall project administration and coordination of TSSWCB Project (09-54) and ensuring that project personnel produce QA/QC data according to the

project's QAPP. Also responsible for ensuring that proper cooperation and coordination are maintained between project personnel on project (09-54) *Assessment of Contact Recreation Use Impairments and Watershed Planning for Big Cypress Creek and Tributaries (Hart and Tankersley Creeks)* and (09-55) *Modeling Support and Bacterial Source Tracking for Big Cypress Creek Bacteria Assessment*.

Lee Thomas, Project Manager, NETMWD

Responsible for overall project management activities related to TSSWCB Project (09-54). Oversees field data collection and ensures that field staff collect QA/QC data according to the QAPP established for Project (09-54). Responsible for the timely collection and delivery of QA water quality data, water samples, fecal samples and watershed inventory data to contractors and sub-contractors on TSSWCB projects (09-55). Monitors and assesses the quality of work. Coordinates attendance at conference calls, training, meetings, and related project activities with the TSSWCB.

Ana-Lab Corporation – Responsible for conducting laboratory analytical analysis on samples received from NETMWD and properly preparing and shipping bacteriological samples to SAML for BST analysis.

Bill Peery, Jr., Executive Vice President, Ana-Lab Corporation

Provides supervision for laboratory procedures and serves as the primary point of contact for all laboratory activity conducted by Ana-Lab Corporation. Responsible for oversight of all operations, ensuring that all QA/QC requirements are met, and documentation related to the analysis is completely and accurately reported.

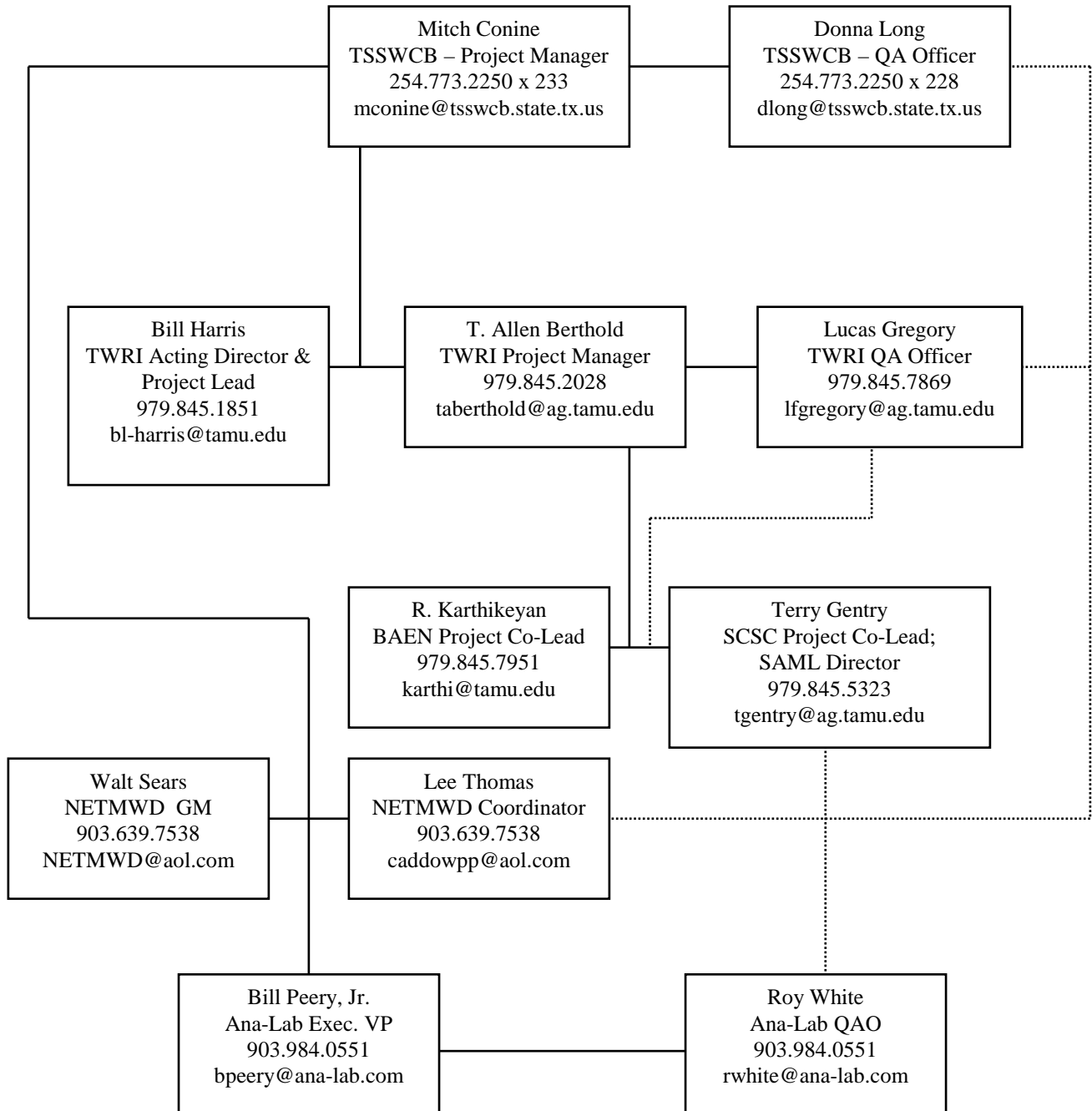
Roy White, QAO, Ana-Lab Corporation

Provides supervision for laboratory procedures. Provides laboratory QA/QC and responsible for updating the laboratory's QAPP. Responsible for ensuring that laboratory personnel involved in generating analytical data have adequate training and a thorough knowledge of the QAPP and all SOPs specific to the analysis or task performed and or supervised. Responsible for making sure QA/QC requirements of this QAPP. Notifies the NETMWD Project Manager and/or SAML Director of circumstances that may adversely affect the quality of data. Enforces corrective actions as required and is responsible for supervision of laboratory personnel involved in generating analytical data for this project.

Responsible for traceability of laboratory standards and reagents, completeness and acceptability of chain of custody forms, maintaining current NELAC Accreditation, ensuring laboratory instrument and calibration data is complete. Ensures that laboratory analysis of QC samples occurs at the required frequency and assists with determination of QC results as they pertain to performance and program specifications. Responsible for the analytical sensitivity of laboratory instrumentation to levels consistent with this QAPP. Performs laboratory bench-level reviews and ensures that all laboratory samples are analyzed for all parameters.

**Figure A4.1 Organization Chart**

Dashed lines indicate communication only



## **Section A5: Problem Definition/Background**

Big Cypress Creek (and its tributaries) are located in the Cypress Creek Basin. The headwaters of Big Cypress Creek originate in southeast Hopkins County. From there, Big Cypress Creek flows east into Lake Cypress Springs and then into Lake Bob Sandlin in Franklin County. After leaving Lake Bob Sandlin, Big Cypress Creek, which forms the county line between Titus and Camp Counties, flows southeast to Lake O' the Pines and then finally to Caddo Lake before entering Louisiana. The watershed is characterized by gently rolling wooded hills and broad, frequently flooded, densely vegetated stream bottoms. Post oak savannah is predominant in the western portion of the basin, while pineywoods are common in the eastern portion.

The Big Cypress Creek watershed, between Lake Bob Sandlin and Lake O' the Pines, encompasses approximately 445 square miles in Camp, Morris, Titus and Upshur Counties. In 1996, Big Cypress Creek (Segment 0404) was placed on the *Texas 303(d) List* for having bacteria levels that exceed water quality standards. In 2000, Tankersley Creek (Segment 0404B) was placed on the *303(d) List* for bacteria, and in 2006, Hart Creek (Segment 0404C) was placed on the *303(d) List* for bacteria. Other tributaries to Big Cypress Creek are not currently impaired for bacteria, but they are likely contributing some degree of bacteria loading to the impaired reaches of Big Cypress Creek.

The focus of this project will be on bacterial water quality issues in Segments 0404, 0404B, and 0404C. Additionally, Segment 0404 is the contributing watershed to Lake O' the Pines (Segment 0403) and is covered by *One Total Maximum Daily Load for Dissolved Oxygen in Lake O' the Pines (Segment 0403)*. The TMDL was adopted by the TCEQ on April 12, 2006, approved by the TSSWCB on March 23, 2006 and approved by the EPA on June 7, 2006. The TMDL determined that low dissolved oxygen concentrations in the reservoir are due to high rates of photosynthesis and respiration in aquatic vegetation and that phosphorus is the limiting nutrient during the critical conditions. The TMDL determined that a 56% reduction in total phosphorus loading is needed to restore water quality. An Implementation Plan (I-Plan) was developed to reduce phosphorus loadings from the contributing watershed. Implementation strategies were identified for point source dischargers (total phosphorus effluent limits), animal feeding operations (BMPs to reduce runoff of sediment and nutrients from poultry litter application sites and dairies), forestry operations (BMPs to reduce runoff of sediment and nutrients), and other sources (on-site sewage facilities, boat sewage disposal, sites permitted for land application of domestic sewage sludge). On July 9, 2008, the TCEQ approved the *I-Plan for One TMDL for Dissolved Oxygen in Lake O' the Pines*. The TSSWCB approved the I-Plan on July 17, 2008. It is anticipated that many of the implementation strategies designed to reduce phosphorus loadings will also have a positive impact on reducing bacteria loadings to Big Cypress Creek.

Through the Lake O' the Pines TMDL process, watershed stakeholders have become extremely familiar with water quality rules and regulations, as well as, approaches to

watershed planning. As such, local stakeholders have already expressed interest in taking an active role in addressing the bacteria impairments.

Land use in the watershed is predominantly cropland and pasture (about 48%) and forest (about 40%). During periods of rainfall, which averages approximately 46 inches annually, bacteria originating from aquatic birds and mammals, livestock, inadequately treated sewage, and/or failing septic systems may be washed into the streams and have the potential to impede recreational use of the waterbodies. Bacterial indicators, such as *E. coli*, may remain in the streams at levels exceeding established criteria and can be measured well after a rain event has occurred. These microorganisms are normally found in wastes of warm-blooded animals and are generally not harmful to human health, but may indicate the presence of pathogens that can cause disease.

Lake O' the Pines and other waterbodies in its watershed are extremely important to the surrounding region. Lake O' the Pines provides drinking water for 7 cities and towns, numerous rural water districts, and several steel manufacturing and electric generating companies. In addition, the City of Longview (population 70,000) will be using the lake as a drinking water source in the near future. The lake is an important resource to the timber industry and to agricultural enterprises such as the poultry industry, dairies, cow/calf operations, and for irrigation. Recreation and tourism are significant sources of income for residents of the watershed. Boating and fishing for trophy bass, catfish, and crappie lure large numbers of recreational users to the watershed each year.

The TCEQ and the TSSWCB established a joint, technical Task Force on Bacteria TMDLs in September 2006 charged with making recommendations on cost-effective and time-efficient bacteria TMDL development methodologies. The Task Force recommended the use of a three-tier approach that is designed to be scientifically credible and accountable to watershed stakeholders. The tiers move through increasingly aggressive levels of data collection and analysis in order to achieve stakeholder consensus on needed load reductions and strategies to achieve those reductions. In June 2007, the TCEQ and the TSSWCB adopted the principles and general process recommended by the Task Force and directed agency staff to incorporate the principles of the recommendations into an updated joint-agency TMDL guidance document.

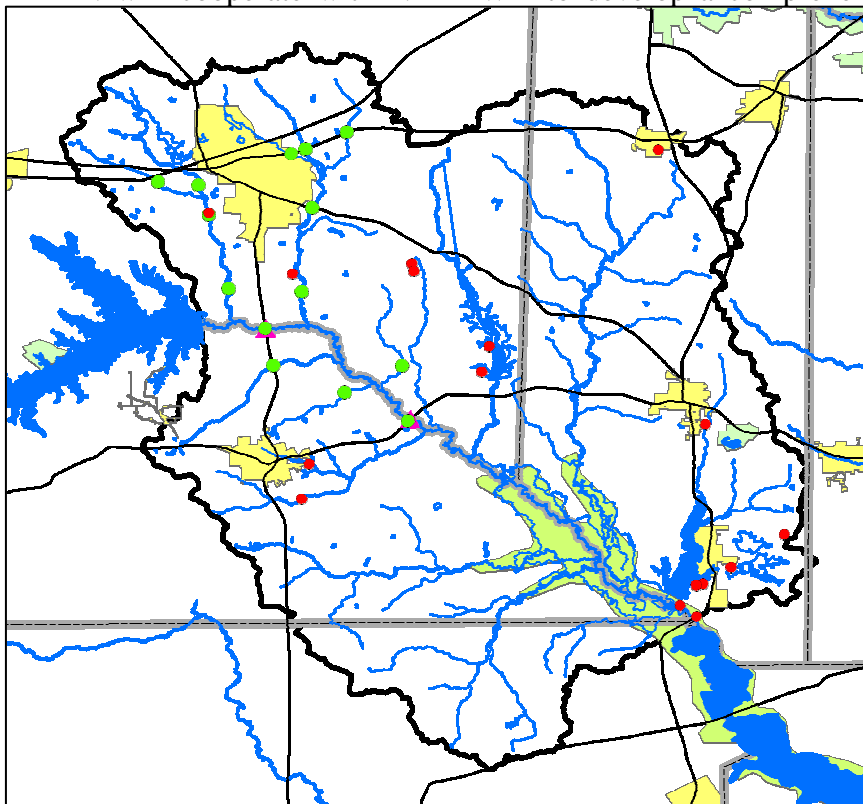
Major revisions to the Texas Surface Water Quality Standards are being drafted by TCEQ, including the establishment of numeric nutrient criteria for reservoirs and modifications to contact recreation use and bacteria criteria. As part of this process, TCEQ is developing procedures for conducting recreational Use Attainability Analyses (UAAs). In order for a new category of recreational use or a different bacteria water quality standard to be applied to a waterbody, a recreational UAA will need to be conducted. TCEQ and TSSWCB have collaborated on developing a list of priority waterbodies for conducting recreational UAAs. Segments 0404, 0404B, and 0404C are on that list.

In accordance with the *Memorandum of Agreement Between the TCEQ and the TSSWCB Regarding TMDLs, I-Plans, and Watershed Protection Plans*, the TSSWCB has agreed to take the lead role in addressing the bacteria impairments in the study area. Through this and associated projects, the TSSWCB and NETMWD will work with local stakeholders to progress through the data collection and analysis components of the first two tiers of the Task Force recommended three-tier approach. The goal is to remove the waterbodies in the study area from the *303(d) List*; however, the mechanism is not predetermined. At the end of this two-year assessment project, possible outcomes include: 1) waterbodies are achieving current water quality standards, 2) waterbodies are achieving revised water quality standards, based on TCEQ triennial review process, 3) adequate data exists to support a UAA to change water quality standards, 4) adequate data exists to develop a Watershed Protection Plan (WPP), or 5) adequate data exists to develop a TMDL and I-Plan for TCEQ adoption.

## Section A6: Project Goals and Task Description

In order to communicate project goals, activities, results and accomplishments to affected parties, TWRI, SAML, and BAEN will participate in public stakeholder meetings as needed. At a minimum, public stakeholder meetings shall consist of an organizational/kick-off meeting, a source survey design meeting, a meeting presenting results from initial data analysis and the GIS inventory, a Texas Watershed Steward Program workshop, two project update meetings during the middle of the project, a meeting presenting data analysis results, and a meeting presenting final technical reports.

BAEN will cooperate with NETMWD to develop a comprehensive GIS inventory for the



study area through TSSWCB project 09-54 *Assessment of Contact Recreation Use Impairments and Watershed Planning for Big Cypress Creek and Tributaries (Hart and Tankersley Creeks).*

TSSWCB, in cooperation with the Spatial Sciences Lab (SSL), will provide BAEN a current land use classification, based on 2004-2006 imagery, for the study area through TSSWCB project 08-52 *Classification of Current Land Use/Land Cover for Certain Watersheds Where TMDLs or WPPs Are In Development.*

**Figure A6.1. Big Cypress Creek Watershed**

In order to apply knowledge gained through TSSWCB project 07-06, *Fate and Transport of E. coli in Rural Texas Landscapes and Streams*, BAEN will assist NETMWD in designing a watershed source survey, to be conducted through TSSWCB project 09-54, that better characterizes the possible sources of bacteria loadings in the study area. SAML will also assist NETMWD in designing the watershed source survey.

To provide sufficient water quality data to characterize bacteria loadings in the study area, NETMWD, through TSSWCB project 09-54, will conduct routine ambient monitoring,

effluent monitoring, and biased-flow monitoring under high flow (storm event influenced) conditions.

TWRI will develop a Quality Assurance Project Plan (QAPP) to ensure data of known and acceptable quality are generated and used in this project. The QAPP shall be consistent with the *TSSWCB Environmental Data Quality Management Plan*.

To determine bacteria load reductions needed to achieve water quality standards, BAEN, with assistance from NETMWD, will conduct a Load Duration Curve (LDC) analysis of all historic and existing water quality monitoring data from the study area. LDCs will be developed for at least one critical index site per segment. LDCs shall be consistent with 1) EPA's *An Approach for Using Load Duration Curves in the Development of TMDLs*, 2) EPA's *Options for Expressing Daily Loads in TMDLs*, and 3) EPA's *Development of Duration-Curve Based Methods for Quantifying Variability and Change in Watershed Hydrology and Water Quality*. Then, using water quality monitoring data collected by NETMWD through TSSWCB project 09-54, BAEN will refine the developed LDCs.

To estimate loadings from various sources and to identify critical loading areas within the watersheds, BAEN, again with assistance from NETMWD, will conduct watershed modeling for the study area. Utilizing information from the GIS inventory, watershed source survey, and water quality monitoring, and in combination with the LDCs, BAEN will develop a spatially explicit or mass balance model, such as SELECT, for each of the three segments in the study area.

To assess and identify different sources contributing to bacteria loadings, SAML will conduct Bacterial Source Tracking (BST) in the study area. SAML will conduct library-independent BST utilizing the *Bacteroidales* PCR genetic test for human, ruminant, horse, and swine markers. Additionally, SAML will conduct limited library-dependent BST and analyze *E. coli* isolates utilizing the ERIC-PCR and RiboPrinting combination method. This will serve to confirm that the sources of *E. coli* and *Bacteroidales* are comparable and assess the spatial and temporal adequacy of the Texas Known Source Library. The Texas Known Source Library may need to be supplemented with known fecal samples from the study area. NETMWD will provide SAML a subset of water samples collected through TSSWCB project 09-54 for BST. Additionally, NETMWD will collect known fecal samples. Results from the source survey will be used by SAML to make appropriate adjustments to the BST sampling design and to assess the adequacy of the Texas Known Source Library. SAML will work with BAEN to integrate BST results into the model, to the extent possible, and address and reconcile discrepancies between BST and modeling results.

## **Task 1: Project Administration**

**Objective:** To effectively administer, coordinate, and monitor all work performed under this project including technical and financial supervision and preparation of status reports.

**Subtask 1.1:** TWRI will prepare electronic quarterly progress reports (QPRs) for submission to the TSSWCB. QPRs shall document all activities performed within a quarter and shall be submitted by the 15<sup>th</sup> of December, March, June and September. QPRs shall be posted to the project website and provided to all project partners. (Start Date: Month 1; Completion Date: Month 24)

**Subtask 1.2:** TWRI will perform accounting functions for project funds and will submit appropriate Reimbursement Forms to TSSWCB at least quarterly. (Start Date: Month 1; Completion Date: Month 24)

**Subtask 1.3:** TWRI will host, and BAEN and SAML will participate in, coordination meetings or conference calls with TSSWCB, and any project partners as appropriate, at least quarterly to discuss project activities, project schedule, communication needs, deliverables and other requirements. Coordination with TSSWCB project 09-54, *Assessment of Contact Recreation Use Impairments and Watershed Planning for Big Cypress Creek and Tributaries (Hart and Tankersley Creek)*, will be especially critical to achieve project goals. (Start Date: Month 1; Completion Date: Month 24)

**Subtask 1.4:** In order to communicate project goals, activities, results and accomplishments to affected parties, TWRI, BAEN, and SAML will participate in public stakeholder meetings as needed. At a minimum, public stakeholder meetings shall consist of an organizational/kick-off meeting (month 3), a source survey design meeting (month 4), a meeting presenting results from initial LDCs and the GIS inventory (month 6), Texas Watershed Steward Program workshop (month 9), two project update meetings (months 12 and 18), a meeting presenting data analysis results (month 21), and a meeting presenting final technical reports (month 24). (Start Date: Month 1; Completion Date: Month 24)

**Subtask 1.5:** TWRI will develop and disseminate educational materials to watershed stakeholders, including, but not limited to, flyers, brochures, letters, and news releases. BAEN, SAML, and NETMWD (through TSSWCB project 09-54), shall contribute content matter for educational materials as appropriate. (Start Date: Month 1; Completion Date: Month 24)

**Subtask 1.6:** TWRI will develop (Months 1-3), host and maintain (Months 4-24) an internet webpage for the dissemination of project information. BAEN, SAML, and NETMWD (through TSSWCB project 09-54), shall contribute content matter for the webpage as appropriate. (Start Date: Month 1; Completion Date: Month 24)

### **Deliverables**

- Quarterly Progress Reports in electronic format
- Reimbursement Forms in either electronic or hard copy format
- Educational materials, as developed and disseminated
- Project webpage

### **Task 2. Quality Assurance**

**Objective:** To develop and implement DQOs and quality assurance/quality control (QA/QC) activities to ensure data of known and acceptable quality are generated through this project.

**Subtask 2.1:** TWRI will develop a QAPP for activities in Tasks 3 and 4 consistent with *EPA Requirements for Quality Assurance Project Plans (QA/R-5)* (May 2006) and the *TSSWCB Environmental Data Quality Management Plan* (August 2007). (Start Date: Month 1; Completion Date: Month 2)

**Subtask 2.2:** TWRI will submit revisions and necessary amendments to the QAPP as needed. (Start Date: Month 3; Completion Date: Month 24)

### **Deliverables**

- QAPP for Task 3 approved by TSSWCB in both electronic and hard copy formats
- Approved revisions and amendments to QAPP
- Data of known and acceptable quality as reported through Task 3 & 4

### **Task 3: Bacterial Source Tracking**

**Objective:** To conduct Bacterial Source Tracking to assess and identify different sources contributing to bacteria loadings.

**Subtask 3.1:** SAML will conduct library-independent BST on 50-100 water samples per segment utilizing the *Bacteroidales* PCR genetic test for human, ruminant, horse, and swine markers. The number of samples may be adjusted depending on the size of each watershed in the study area and the complexity of sources as identified in the source survey (Subtask 3.4). Budgeted number of samples is 75 from each of Hart and Tankersley Creeks and 100 from Big Cypress Creek (main stem) for a total of 250. Specific genetic markers for various animal sources are continually being developed by the scientific community and as new markers are identified, they should be included in this analysis, as the budget allows. Water samples for this subtask shall be a subset of those collected by NETMWD through TSSWCB project 09-54. (Start Date: Month 3; Completion Date: Month 21)

**Subtask 3.2:** SAML will conduct limited library-dependent BST and analyze *E. coli* isolates from 50-100 water samples (1 isolate per water sample) from across the study area utilizing the ERIC-PCR and RiboPrinting combination method. Budgeted number of samples is 100. This will serve to 1) confirm that the sources of *E. coli* and *Bacteroidales* are comparable and 2) assess the spatial and temporal adequacy of the Texas Known Source Library. Water samples for this subtask shall be a subset of those collected by NETMWD through TSSWCB project 09-54. (Start Date: Month 3; Completion Date: Month 21)

**Subtask 3.3:** SAML will add up to 30 known source fecal samples (1-2 isolates per fecal sample) to the Texas Known Source Library. Fecal samples will be added to the BST library utilizing the ERIC-PCR and RiboPrinting combination method. Samples for this subtask shall be collected by NETMWD through TSSWCB project 09-54. (Start Date: Month 3; Completion Date: Month 21)

**Subtask 3.4:** SAML will assist NETMWD in designing a watershed source survey (also known as a sanitary survey), to be conducted through TSSWCB project 09-54, that better characterizes possible sources of bacteria loadings in the study area. Results from the source survey will be used by SAML to make appropriate adjustments to the BST sampling design and assess the adequacy of the Texas Known Source Library. (Start Date: Month 1; Completion Date: Month 15)

**Subtask 3.5:** BAEN will conduct watershed modeling for the study area (Task 4). SAML will work with BAEN to 1) integrate BST results into the model, to the extent possible, and 2) address and reconcile discrepancies between BST and modeling results. (Start Date: Month 7; Completion Date: Month 21)

**Deliverables:**

- Technical Report detailing the results of Bacterial Source Tracking

**Task 4: Data Analysis and Watershed Modeling**

**Objective:** To develop a comprehensive GIS inventory for the study area and to assess the possible sources of bacteria loadings by conducting a watershed source survey. To analyze and interpret data using Load Duration Curves and spatially explicit modeling to determine bacteria load reductions needed to achieve water quality standards and estimate loadings from various sources.

**Subtask 4.1:** BAEN will cooperate with NETMWD to develop a comprehensive GIS inventory for the study area through TSSWCB project 09-54. Data will be collected by NETMWD and should include the most recent information available on land use, elevation, soils, stream networks, reservoirs, roads, public parks, municipalities and

satellite imagery or aerial photography. Locations of SWQM stations, USGS gages, public access points to the waterbodies, floodwater-retarding structures, wetlands, TPDES permittees (including WWTFs, CAFOs and MS4s), and subdivisions should also be included. Sites permitted for land application of sewage sludge and septage should be included. Locations of possible bacteria sources, identified in the source survey, should be incorporated. The cumulative impact of TSSWCB-certified WQMPs on the management of agricultural and silvicultural lands should be documented. BAEN will be responsible for incorporating these data into a GIS, creating maps for stakeholder meetings and utilizing this information for SELECT modeling. (Start Date: Month 1; Completion Date: Month 3)

**Subtask 4.2:** TSSWCB, in coordination with the SSL, will provide BAEN a current land use classification for the study area through TSSWCB project 08-52, *Classification of Current Land Use/Land Cover for Certain Watersheds Where TMDLs or WPPs Are In Development*. (Start Date: Month 1; Completion Date: Month 3)

**Subtask 4.3:** In order to apply knowledge gained through TSSWCB project 07-06, *Fate and Transport of E. coli in Rural Texas Landscapes and Streams*, BAEN will assist NETMWD in designing a watershed source survey, to be conducted through TSSWCB project 09-54, that better characterizes the possible sources of bacteria loadings in the study area. (Start Date: Month 1; Completion Date: Month 4)

**Subtask 4.4:** BAEN, with assistance from NETMWD through TSSWCB project 09-54, will conduct a LDC analysis of all historic and existing water quality monitoring data from the study area. LDCs will be developed for one critical index site per segment (0404, 0404B, 0404C). LDCs shall be consistent with 1) EPA's *An Approach for Using Load Duration Curves in the Development of TMDLs*, 2) EPA's *Options for Expressing Daily Loads in TMDLs*, and 3) EPA's *Development of Duration-Curve Based Methods for Quantifying Variability and Change in Watershed Hydrology and Water Quality*. LDC development will be completed using available USGS flow gage data and SWAT generated flow data as reported in the Lake O' the Pines TMDL. (Start Date: Month 3; Completion Date: Month 6)

**Subtask 4.5:** Using water quality monitoring data collected by NETMWD through TSSWCB project 09-54, BAEN, with assistance from NETMWD, will refine LDCs developed in subtask 4.4. LDCs will be used to determine bacteria load reductions needed to achieve water quality standards. (Start Date: Month 7; Completion Date: Month 21)

**Subtask 4.6:** BAEN, with assistance from NETMWD through TSSWCB project 09-54, will conduct watershed modeling for the study area. Utilizing information from the GIS inventory (Subtask 4.1), the source survey (Subtask 4.3), and water quality monitoring (TSSWCB project 09-54), and in combination with LDCs from Subtasks 4.4-4.5, BAEN will develop a spatially explicit or mass balance model, such as

SELECT, for each of the three segments in the study area. The model will be conducted on the entire Big Cypress Creek watershed; SELECT output subsets highlighting the individual impacts of Hart and Tankersly Creeks will also be provided. Modeling will be used to estimate loadings from various sources and to identify critical loading areas within the watersheds. (Start Date: Month 7; Completion Date: Month 21)

### Deliverables

- Draft Technical Report detailing preliminary LDC analysis
- Technical Report detailing final LDC analysis
- Technical Report describing watershed modeling results

**Table A6.1. Project Plan Milestones**

Task	Project Milestones	Agency	Start	End
1.1	Prepares and submit quarterly reports to TSSWCB & project participants	TWRI	June 09	May 11
1.2	Perform accounting functions and submit Reimbursement Forms to TSSWCB	TWRI	June 09	May 11
1.3	Host and participate in coordination meetings with TSSWCB and project partners	TWRI; BAEN, SAML	June 09	May 11
1.4	Participate in public stakeholder meetings as needed	TWRI, BAEN, SAML	June 09	May 11
1.5	Develop & disseminate educational materials to stakeholders	TWRI	June 09	May 11
1.6	Develop, host & maintain project website	TWRI	June 09	May 11
2.1	Develop a QAPP for activities in Task 3 & 4	TWRI	June 09	July 09
2.2	TWRI will submit revisions and necessary amendments to the QAPP as needed.	TWRI	Aug 09	May 11
3.1	Conduct library-independent BST on 50-100 water samples per segment	SAML	Aug 09	Feb 11
3.2	Conduct library-dependent BST on 50-100 water samples from study area	SAML	Aug 09	Feb 11
3.3	Add up to 30 known source fecal samples to Texas Known Source Library	SAML	Aug 09	Feb 11
3.4	Assist NETMWD in designing a watershed source survey	SAML	June 09	Aug 10
3.5	Work with BAEN to integrate BST into modeling and resolve discrepancies	SAML	Dec 09	Feb 11
4.1	Cooperate with NETMWD to develop GIS inventory for the study area	BAEN	June 09	Aug 09
4.2	Provide BAEN a current land use classification	SSL, TSSWCB	June 09	Aug 09
4.3	Assist NETMWD in designing a watershed source survey	BAEN	June 09	Sept 09
4.4	Conduct a LDC analysis of all historic and existing water quality monitoring data from the study area	BAEN	Aug 09	Nov 09
4.5	Refine LDCs developed in subtask 4.4. LDCs using data collected by NETMWD in project 09-54	BAEN	Dec 09	Feb 11
4.6	Conduct watershed modeling for the study area	BAEN	Dec 09	Feb 11

### **Bacterial Source Tracking Descriptions**

#### **Identification of Sources**

New data, of known and specified quality, will be collected and analyzed to differentiate and quantify the relative contributions of livestock, wildlife, and other human and animal *E. coli* sources to Big Cypress Creek and its tributaries Hart and Tankersley Creeks. This assessment and differentiation between bacteria sources will utilize the BST Texas Known Source Library coordinated by AgriLife El Paso. The library contains diverse *E. coli* isolates that were selected after screening over 4,400 isolates by genetic fingerprinting to exclude identical isolates from the same sample and include isolates with unique genetic fingerprints. This project will provide sufficient documentation of the data and technical analyses conducted that will aid the project staff in communicating the assessment results to watershed stakeholders and TSSWCB.

Fifty to one-hundred *E. coli* isolates from 50-100 different water samples (1 isolate per water sample) collected from across the study area will be analyzed by SAML using the (ERIC-PCR) and RiboPrinting BST methods described below and compared with isolates from the previously developed Texas Known Source Library. Additionally, 50-100 water samples collected from each of the three segments will be analyzed by SAML for *Bacteroidales* PCR markers (general, human, ruminant, swine, equine and others as they become available). An experimental approach flow diagram is presented in Figure A6.2.

NETMWD and Ana-Lab will be responsible for collecting water samples through TSSWCB project 09-54 and delivering a subset of those samples to SAML (see Table A6.2 for sampling locations). Ana-Lab will be responsible for pre-processing of water samples for *E. coli* isolations and *Bacteroidales* PCR. *E. coli* will be isolated by SAML from the samples using standard microbiological methods as previously used in TSSWCB and TCEQ BST projects. *E. coli* will be quantified and then isolated from water samples using EPA Method 1603 and modified membrane Thermotolerant *E. coli* (mTEC) medium. The modified mTEC method is a single-step method that uses one medium and does not require testing using any other substrate.

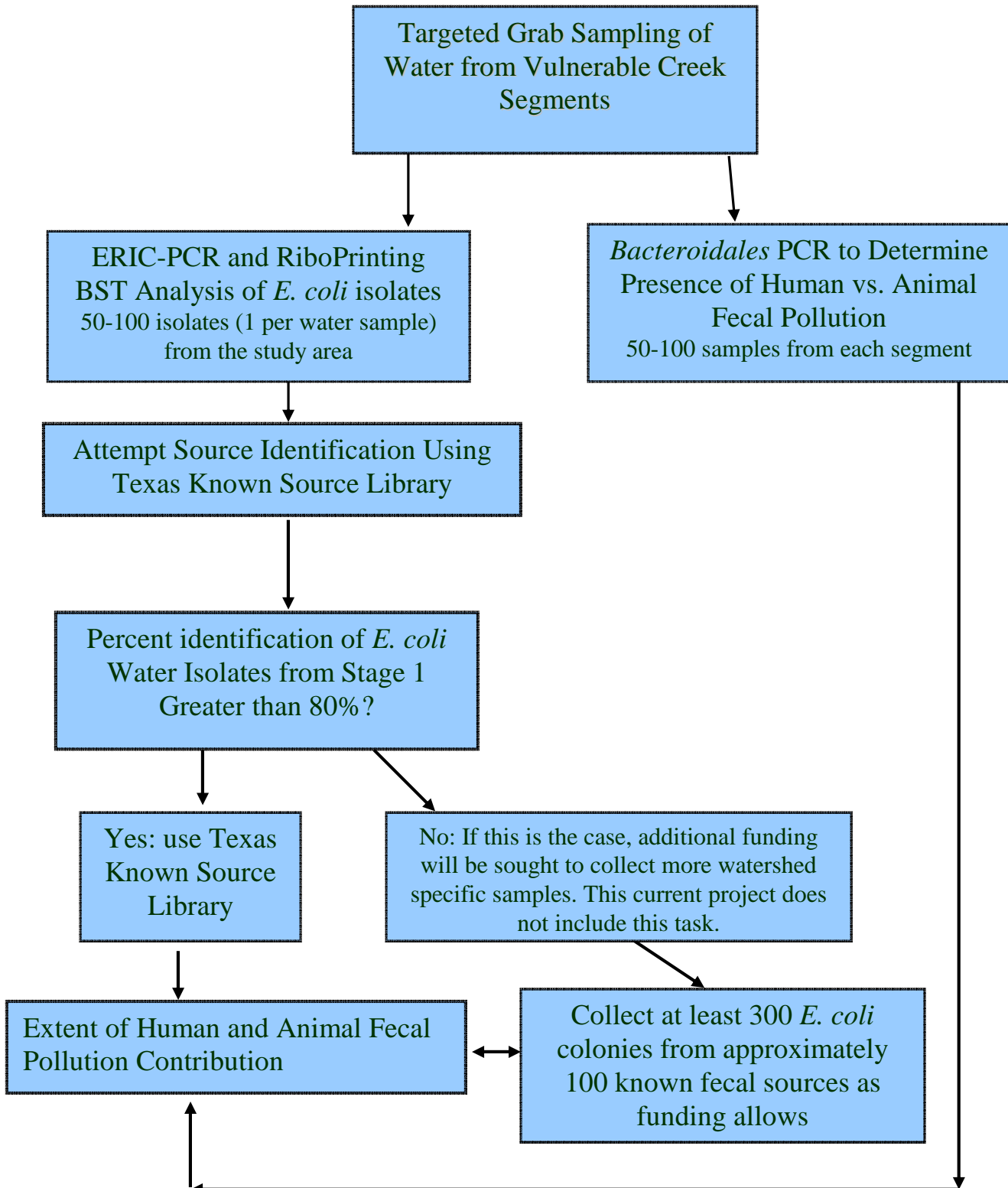


Figure A6.2. Flow Diagram of Experimental Approach for BST

### **Limited Library Dependant BST**

Enterobacterial repetitive intergenic consensus sequence polymerase chain reaction (ERIC-PCR), a type of rep-PCR, has moderately high ability to resolve different closely related bacterial strains (Versalovic, Schneider et al. 1994). Consumable costs for ERIC-PCR are inexpensive and labor costs for sample processing and data analyses are moderate. ERIC-PCR is a genetic fingerprinting method used in previous BST studies as well as many microbial ecology and epidemiological studies. ERIC elements are repeat DNA sequences found in varying numbers and locations in the genomes of different bacteria such as *E. coli*. The PCR is used to amplify the DNA regions between adjacent ERIC elements. This generates a DNA banding pattern or fingerprint which looks similar to a barcode pattern. Different strains of *E. coli* bacteria have different numbers and locations of ERIC elements in their bacterial genomes, and therefore, have different ERIC-PCR fingerprints. ERIC-PCR is useful as a screening technique for library development because of its moderate cost and moderately high ability to resolve different strains of the same species of bacteria. Though rep-PCR banding patterns for isolates tend to be generally stable, differences in fingerprint image processing and PCR protocols between laboratories may result in reduced between-laboratory reproducibility and pose a challenge to generating a composite library in multiple laboratories. Rigorous QC and QA, standardized protocols for PCR and image processing, and adequate training of personnel is crucial for generation of comparable data (Bacteria TMDL Task Force Final Report; TWRI TR-341).

Ribotyping is a genetic fingerprinting method used in previous BST studies and many microbial ecology and epidemiological studies. In general, an endonuclease enzyme (*Hind* III) selectively cuts *E. coli* DNA wherever it recognizes a specific DNA sequence. The resulting DNA fragments are separated by size and probed for fragments containing particular conserved ribosomal RNA gene sequences, which results in DNA banding patterns or fingerprints that look similar to barcodes. Different strains of *E. coli* bacteria have differences in their DNA sequences and different numbers and locations of enzyme cutting sites, and therefore have different ribotyping fingerprints. The DuPont Qualicon RiboPrinter Microbial Characterization System allows automation of the ribotyping ('RiboPrinting').

A total of 50-100 *E. coli* isolates obtained from ambient water samples from across the study area will be characterized using ERIC-PCR and RiboPrinting. DNA patterns of those isolates will be compared to the Texas Known Source Library of *E. coli* isolates from known animal and human sources collected throughout Texas. Water isolates will be identified to cattle, other livestock, avian and non-avian wildlife, domestic sewage, and pet sources (six-way split), as well as a broader three-way split of livestock, domestic sewage and wildlife.

### **Library Independent BST**

PCR genetic testing for *Bacteroides* fecal bacteria will be performed by SAML to determine the source of the fecal pollution. The *Bacteroidales* PCR method is a culture-independent molecular method which targets genetic markers of *Bacteroidales* and *Prevotella* spp. fecal bacteria that are specific to humans, ruminants (including cattle and deer), pigs, and horses [Bernhard, A. E. and K. G. Field (2000). "A PCR assay to discriminate human and ruminant

feces on the basis of host differences in *Bacteroides-Prevotella* genes encoding 16S rRNA.” *Appl Environ Microbiol* 66(10): 4571-4574; Dick, L. K., A. E. Bernhard, et al. (2005). “Host distributions of uncultivated fecal *Bacteroidales* bacteria reveal genetic markers for fecal source identification.” *Appl Environ Microbiol* 71(6): 3184-3191]. The method has high specificity and moderate sensitivity [Field, K. G., E. C. Chern, et al. (2003). “A comparative study of culture-independent, library-independent genotypic methods of fecal source tracking.” *J Water Health* 1(4): 181-94]. For this method, 100 ml water samples are concentrated by filtration, DNA extracted from the concentrate and purified, and aliquots of the purified DNA analyzed by PCR. For pre-processing of water samples for *Bacteroidales* PCR, SAML will filter the water samples, place the filters in DNA lysis buffer and freeze at -80° C until analysis. At the time of analysis, SAML will extract and purify DNA from the filters. DNA extracted from the water samples will be tested for the general, human, ruminant (including cattle and deer), pig (including feral hogs), and horse fecal markers. Results are typically expressed as presence/absence of the host-specific genetic markers; therefore, this method is not quantitative.

**Table A6.2. Sampling locations in the Big Cypress Creek watershed**

Station ID – Temporary/ TCEQ ID	Site Description	Latitude (N)	Longitude (W)	Start Date	End Date <sup>1</sup>	Estimated Sampling Frequency (per month)	
						Routine <sup>2</sup>	Storm <sup>3</sup>
DC01	Dragoo Creek at SW 35th Street	33.15724	-95.02693	11/01/2009	08/31/2011	2	0.33
10264	Tankersley Creek at FM 899	33.155369	-95.003694	11/01/2009	08/31/2011	2	0.33
10263	Tankersley Creek at FM 127	33.138371	-94.997661	11/01/2009	08/31/2011	2	0.33
10261	Tankersley Creek at FM 3417	33.095894	-94.986475	11/01/2009	08/31/2011	2	0.33
10310	Big Cypress Creek at US 271	33.072987	-94.965431	11/01/2009	08/31/2011	2	0.33
16454	Walkers Creek at US 271	33.051731	-94.960789	11/01/2009	08/31/2011	2	0.33
EV01	Evans Creek at US 67	33.18587	-94.91866	11/01/2009	08/31/2011	2	0.33
HC01	Hayes Creek at US 67	33.17326	-94.95063	11/01/2009	08/31/2011	2	0.33
10273	Hart Creek at US 67	33.176048	-94.942108	11/01/2009	08/31/2011	2	0.33
10272	Hart Creek at SH 49	33.142319	-94.938389	11/01/2009	08/31/2011	2	0.33
10266	Hart Creek at Titus CR SE-12	33.094185	-94.944356	11/01/2009	08/31/2011	2	0.33
UT01	Unnamed trib of BCC at Dukes Chapel Rd	33.03612	-94.91969	11/01/2009	08/31/2011	2	0.33
PB01	Prairie Branch at FM 2348	33.05159	-94.8869	11/01/2009	08/31/2011	2	0.33
10308	Big Cypress Creek at SH 11	33.01973	-94.883558	11/01/2009	08/31/2011	2	0.33
16467	City of Mt. Pleasant WWTP	33.10416	-94.948156	11/01/2009	08/31/2011	2	0.33
16468	Pilgrims Pride Processing WWTP	33.139095	-94.995322	11/01/2009	08/31/2011	2	0.33

### Modeling Analysis Descriptions

#### Statistical Models

- Spatially Explicit Load Enrichment Calculation Tool (SELECT)
- Load duration curve

#### Spatially Explicit Load Enrichment Calculation Tool (SELECT)

The Center for TMDL and Watershed Studies at Virginia Tech has been involved in TMDL development for bacteria impairments. The Center personnel developed a systematic process for source characterization that includes the following steps:

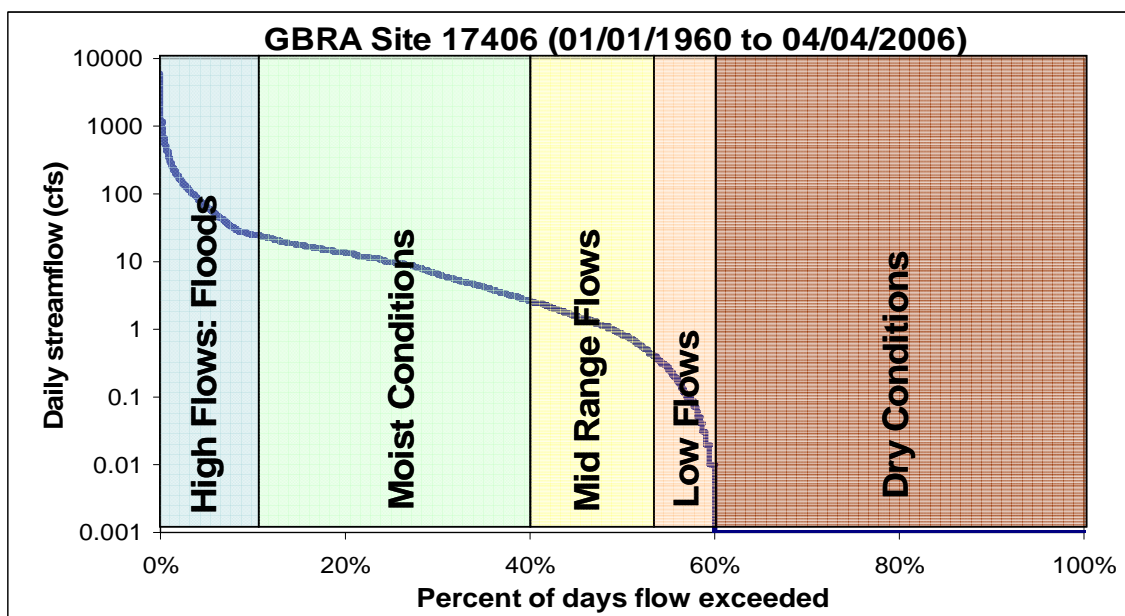
- inventorying bacterial sources (including livestock, wildlife, humans, and pets);
- distributing estimated loads to the land as a function of land use and source type; and
- generating bacterial load input parameters for watershed-scale simulation models.

This process provides a consistent approach that is necessary to develop comprehensive bacteria TMDLs. The Center personnel developed a software tool, the Bacteria Source Load Calculator (BSLC), to assist with the bacterial source characterization process and to automate the creation of input files for water quality modeling (Zeckoski, et al., 2005); however, BSLC does not spatially reference the sources. A spatially-explicit tool, Spatially Explicit Load Enrichment Calculation Tool (SELECT) is being developed by Spatial Sciences Laboratory and Biological and Agricultural Engineering, TAMU to calculate contaminant-loads resulting from various sources within a watershed. SELECT spatially references the sources, and is being developed under ArcGIS 9 environment. SELECT will calculate and allocate pathogen loading to a stream from various sources within a watershed. All loads will be spatially referenced. In order to allocate the *E. coli* load throughout the selected watershed, estimations of the source contributions will be made. This in turn allows the sources and locations to be ranked according to their potential contribution within watershed. The populations of agricultural animals, wildlife, and domestic pets will be calculated and distributed throughout each watershed according to appropriate land use. Furthermore, point sources such as wastewater treatment facilities (WWTFs) will be identified and their contribution quantified based on flow and outflow concentration. Septic system contribution will also be estimated based on criteria including distance to a stream, soil type, failure rate, and age of system. Once the watershed profile is developed for each potential source, the information can be aggregated to the sub-watershed level to identify the top contributing areas in each of the five selected watersheds.

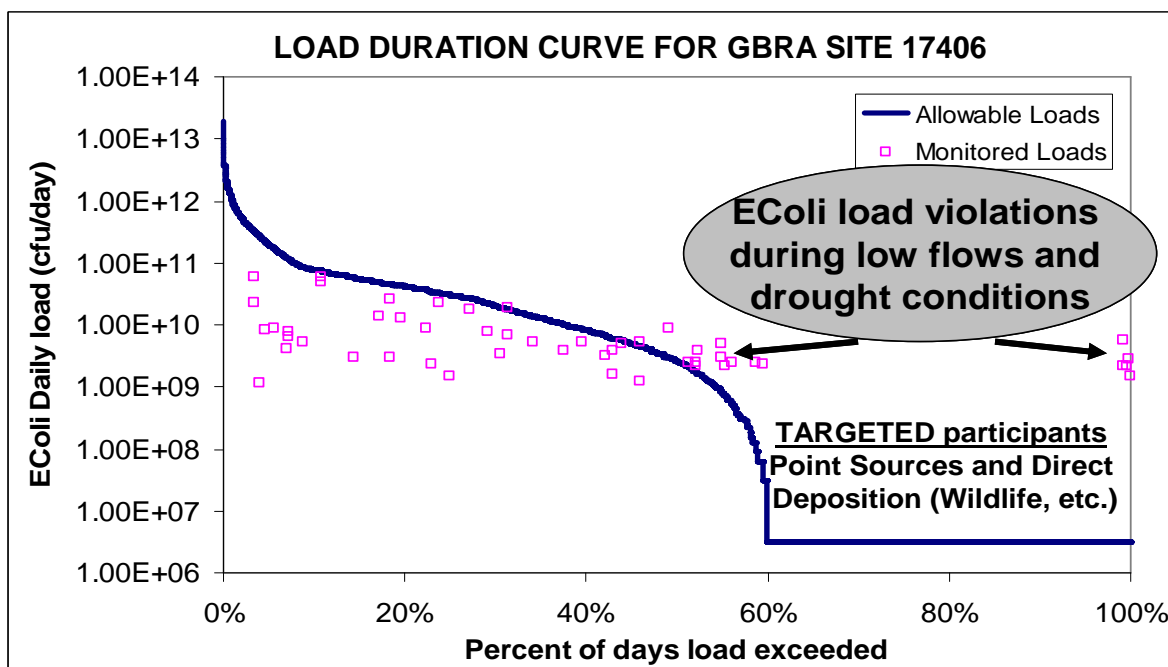
### **Load duration Curve**

This is a simple and an effective first-step methodology to obtain data-based TMDLs (Cleland, 2003; Stiles, 2001). A duration curve is a graph that illustrates the percentage of time during which a given parameter's value is equaled or exceeded. For example, a flow duration curve (FDC) (Figure A6.3) uses the hydrograph of the observed stream flows to calculate and depict the percentage of time the flows are equaled or exceeded.

A load duration curve (LDC) (Figure A6.4), which is related to the FDC, shows the corresponding relationship between the contaminant loadings and stream flow conditions at the monitoring site. In this manner, it assists in determining patterns in pollution loading (point sources, non point sources, erosion, etc.) depending on the streamflow conditions. Based on the observed patterns, specific restoration plans can be implemented that target a particular kind of pollutant source. For example, if the pollutant loads exceed the allowable loads (see Figure A6.4) for low stream flow regimes, then the point sources such as WWTFs and direct deposition sources (wildlife, livestock) should be targeted for the restoration plans. Another main advantage of the LDC method is that it can also be used to evaluate the current impairment as some percent of samples which exceed the standard, and therefore it allows for the rapid development of TMDLs (Stiles, 2001).



**Figure A6.3. Flow Duration Curve (FDC) for streamflow conditions at GBRA monitoring station 17406 on Plum Creek, near Uhlend, TX.** The flow data at 17406 was obtained from the nearest USGS gage station 8172400, after adjusting for subwatershed aerial contribution during runoff events.



**Figure A6.4. Load Duration Curve for *E. coli* at GBRA monitoring station 17406 on Plum Creek, near Uhlend, TX.** The flow data at 17406 was obtained from the nearest USGS gage station 8172400, after adjusting for subwatershed aerial contribution during runoff events.

## Section A7: Quality Objectives and Criteria

### **BST Analysis**

The project objective is to assess contact recreation use impairments and support watershed planning for Big Cypress Creek and its tributaries, Hart and Tankersley Creeks by conducting BST. The measurement performance specifications to support the project objective are specified in Table A7.1. Laboratory measurement QC requirements and acceptability criteria are provided in Section B5.

**Table A7.1. Measurement Performance Specifications**

Parameter	Method Type	Method	Method Description	Precision Of Laboratory Duplicates <sup>1</sup>	Bias <sup>1</sup>	Percent Complete <sup>2</sup>
<i>E. coli</i> RiboPrinting	DNA/ image matching	EP AREC SOP	RiboPrinting	90% identical	90% correct	90
<i>E. coli</i> ERIC-PCR	DNA/ image matching	EP AREC SOP	ERIC-PCR	90% identical	90% correct	90
<i>Bacteroidales</i> PCR	PCR presence /absence	EP AREC SOP	<i>Bacteroidales</i> PCR	100% agreement	90% correct	90
<i>E. coli</i> isolation	Membrane filter culture on modified mTEC agar	USEPA 1603	Membrane Filter	N/A	N/A	N/A

<sup>1</sup> Bias and laboratory method precision will be determined using isolates from known-source samples in a blind procedure, as discussed in Section B5.

<sup>2</sup> The objective is for 90% of the data to be collected. An additional objective for BST completeness is that sources for 70% of host-specific isolates can be identified.

### **Precision**

The precision of laboratory data is a measure of the reproducibility of a result from repeated analyses. It is strictly defined as a measure of the closeness with which multiple analyses of a given sample agree with each other. Precision is assessed by repeated analyses of a sample. Laboratory precision is assessed by comparing sample/duplicate pairs, in the case of bacterial analysis. Precision results are compared against measurement performance specifications and used during evaluation of analytical performance. Measurement performance specifications for precision are defined in Table A7.1.

The ERIC-PCR and RP BST techniques are qualitative assays, generating two different types of DNA fingerprints. For *Bacteroidales* there is a presence/absence qualitative PCR assay. Precision for ERIC-PCR and RP will be determined using a control strain of *E. coli* (QC101), while fecal DNA from known-source samples will be used for *Bacteroidales* PCR. For ERIC-PCR and RP, the DQO is 90% precision. For *Bacteroidales* PCR the DQO is 100% agreement in marker detection among replicates.

## **Bias**

Bias is a statistical measurement of correctness and includes components of systemic error. A measurement is unbiased when the value reported does not differ from the true value. Performance specifications for bias are specified in Table A7.1. In BST, it is best quantified through RP and ERIC-PCR of *E. coli* and PCR of *Bacteroidales* isolated from known sources as “double-blind” samples selected by a third party. Results are compared against measurement performance specifications and used during evaluation of analytical performance. For the *E. coli* methods the DQO is 90% accuracy for correct identification to library strain or source. For *Bacteroidales* PCR, the DQO is 90% accuracy for the presence/absence of the appropriate markers in known source fecal DNA samples.

An additional element of bias is the absence of contamination. This is determined through the analysis of blank samples of sterile water processed in a manner identical to the sample. Requirements for blank samples are discussed in Section B5.

## **Sensitivity**

Sensitivity is a measure that is used to determine a method's detection limits. The detection limit of quantitative methods is defined as the minimum concentration of a substance that can be measured with a given level of confidence that the analyte concentration is greater than zero (*QA/QC Guidance for Laboratories Performing PCR Analyses on Environmental Samples*. EPA, 2004). For presence/absence methods, the detection limit is the minimum concentration of analyte that produces a positive response with a given level of confidence. The detection limits can be expressed as the minimum number of organisms or of the target sequence copy number in a given volume. Many uncertainties can affect the detection limit; some are the:

- type of target nucleic acid being detected (e.g. DNA, mRNA, tRNA, etc.),
- secondary structure and the GC content of the nucleic acid target molecule,
- matrix from which the organism is located, and
- detection of microbes that are inactivated by physical and chemical disinfectants.

For analyzing environmental samples using PCR, the detection limits can be for the limit of the entire method or the limit of the PCR procedure.

## **Representativeness**

Data collected under this project will be considered representative of ambient water quality conditions. Representativeness is a measure of how accurately a monitoring program reflects the actual water quality conditions typical of receiving water. The representativeness of the data is dependent on 1) the sampling locations, 2) the number of samples collected, 3) the number of years and seasons when sampling is performed, 4) the number of depths sampled, and 5) the sampling procedures. Site selection procedures will assure that the measurement data represent the conditions at the site. The goal for meeting total representation of the waterbody and watershed is tempered by the availability of time, site accessibility, and funding. Representativeness will be measured with the completion of sample collection in

accordance with the approved QAPP. Sample collection is dictated by TSSWCB QAPP 09-54 and conducted by NETMWD.

### **Comparability**

The comparability of the data produced is predetermined by the commitment of the staff to use only approved procedures as described in this QAPP. Comparability is also guaranteed by reporting all ambient, high flow, and QC data for evaluation by others.

### **Completeness**

The completeness of the data is a measure of how much of the data is available for use compared with the total potential data. Ideally, 100% of the data would be available. However, the possibility of unavailable data due to accidents, weather, insufficient sample volume, broken or lost samples, etc. is to be expected. Therefore, it will be a general goal of the project(s) that 90% data completion is achieved.

Should less than 90% data completeness occur, the TWRI PM/QAO will initiate corrective action. Data completeness will be calculated as a percent value and evaluated with the following formula:

$$\% \text{ completeness} = (\text{SV} \times 100) / \text{ST}$$

Where:       SV = number of samples with a valid analytical report  
              ST = total number of samples collected

An additional element of completeness is involved with BST. The sources of *E. coli* isolates which do not match those from a library of known sources cannot be identified. In all BST studies, a source cannot be identified with acceptable confidence for a portion of the *E. coli* isolates. This is a function of 1) the size of the library relative to the true diversity of *E. coli* in the watershed, 2) the ability of the method to distinguish sources with acceptable confidence, and 3) the abundance of *E. coli* strains that colonize multiple sources, and thus cannot be used to uniquely identify a source. It will be a general goal of this project to identify the sources of 80% of the *E. coli* strains isolated from water and 90% of the *Bacteroidales* for the general marker in all samples that have a countable number of *E. coli*.

### **Modeling Analysis**

Faculty in the BAEN Department at TAMU will conduct a phased modeling effort to develop pollutant source and loading information and estimates of needed. The objectives of the water quality modeling for this project are as follows:

- 1) Develop and obtain approval for a QAPP
- 2) Conduct efforts in conjunction with NETMWD to develop a comprehensive GIS inventory for the study area through TSSWCB project 09-54, *Assessment of Contact*

*Recreation Use Impairments and Watershed Planning for Big Cypress Creek and Tributaries (Hart and Tankersley Creeks).*

- 3) Conduct efforts in conjunction with NETMWD to design a watershed source survey, to be conducted through TSSWCB project 09-54, that better characterizes the possible sources of bacteria loadings in the study area.
- 4) Spatially characterize and rank sources of bacteria within the watershed using SELECT, a spatially-explicit Geographic Information System (GIS) methodology. Divide the area into sub-watersheds and identify, quantify and rank pollutant loads from various sources, i.e. agriculture, urban/human, wildlife, and other sources for Big Cypress, Hart and Tankersley Creeks.
- 5) Develop two Load Duration Curves (LDC) to analyze the temporal trends in the observed water quantity and quality data for Big Cypress, Hart and Tankersley Creeks. The first set of LDCs will be developed using currently existing data available from NETMWD and/or TCEQ; the second set of LDCs will be developed using data collected by NETMWD under TSSWCB project 09-54. Obtain an interpolated model to simulate the trends of the monitored data. Evaluate the violations and the required load-reductions for different flow-rate regimes (low, medium, and high flow) using LDC and interpolated model.

**SELECT** – this approach is being developed by SSL and BAEN. It is similar to the BSCL (Zeckoski, et al. 2005) that is used in TMDL development. High quality spatial data (Landuse data developed under TSSWCB Project 08-52, SSURGO soils data, NHD, etc) will be processed and utilized in SELECT approach. Distributions for input parameters for SELECT will be created based on literature values and expert knowledge.

**LDC** – this approach has been utilized in several TMDL projects as an initial screening-tool to evaluate the actual temporal load trends in streams (Cleland, 2003; Stiles, 2001). In cases of violations, it is necessary to determine the required load-reduction in that region near the monitoring station. The load-reductions should be calculated for all flow-regimes of the stream. In order to do this continuous monitoring data will be simulated using the actual monitoring data by regression methods. Uncertainty of the model will be estimated via residual error analysis. The straight line passing through residual error plot should have a slope of zero.

## **Section A8: Special Training Requirements/Certification**

### **BST Analysis**

All personnel involved in sample analyses and statistical analyses have received the appropriate education and training required to adequately perform their duties. No special certifications are required. SAML personnel involved in this project have been trained in the appropriate use of laboratory equipment, laboratory safety, cryogenics safety, and all applicable SOPs. Each laboratory analyst must demonstrate their capability to conduct each test that the analyst performs to the Lab Director. This demonstration of capability is performed before analyzing samples and annually thereafter. Finally, SAML is in the process of becoming NELAC-certified for enumerating *E. coli* in both non-potable and drinking water using USEPA Method 1603.

### **Modeling Analysis**

All BAEN personnel involved in model calibration, validation, and development will have the appropriate education and training required to adequately perform their duties. No special certifications are required.

## Section A9: Documentation and Records

### **BST and Modeling Analysis**

The document and records that describe, specify, report, or certify activities, requirements, procedures, or results for this project and the items and materials that furnish objective evidence of the quality of items or activities are listed in Table A9.1.

**Table A9.1 Project Documents and Records**

<b>Document/Record</b>	<b>Location</b>	<b>Retention</b>	<b>Form**</b>
QAPP, amendments, and appendices	Ana-Lab, SAML, TWRI, TSSWCB	5 years	Paper/Electronic
COC records	SAML	5 years	Paper/Electronic
Modeler's notebooks & electronic files	BAEN	5 years	Paper/Electronic
Corrective action reports	Ana-Lab, SAML, TWRI, TSSWCB	5 years	Paper/Electronic
Bacteriological data log sheet	Ana-Lab, SAML	5 years	Paper/Electronic
Laboratory QA manuals	Ana-Lab, SAML	5 years	Paper/Electronic
Laboratory SOPs	Ana-Lab, SAML	5 years	Paper/Electronic
Instrument raw data files, readings and printouts	Ana-Lab, SAML	5 years	Paper/Electronic
Lab equipment calibration records & maintenance logs	Ana-Lab, SAML	5 years	Paper/Electronic
Lab data reports	Ana-Lab, SAML, TSSWCB	3 years	Paper/Electronic
Progress reports/final report/data	TWRI/TSSWCB	3 years	Paper/Electronic

\*\*Paper/Electronic denotes paper or electronic

Individual laboratory notebooks, which contain printouts of laboratory data and hand written observations and data, are kept by individual analysts at SAML or the SCSC Project Co-Lead for at least five years. When lab notebooks are filled, they are stored for at least five years by the SCSC Project Co-Lead/Laboratory Manager in hardcopy form. The SAML keeps electronic data on personal computers for the duration of the project and then in hardcopy files for 5 years after the project. Chain-Of-Custody Forms (COCs) and attached documents are stored in numerical order in three-ring binders in the SCSC Project Co-Lead/Laboratory Manager's office for at least five years. In addition, the SCSC Project Co-Lead/Laboratory Manager will archive electronic forms of all project data for at least five years on personal computers and fire-resistant cabinets. Lab data reports from the SAML, as included in the final report, and other reports as required, will report test results clearly and accurately.

All records, including modeler's notebooks and electronic files, will be archived by BAEN for at least five years. These records will document model testing, calibration, and evaluation and will include documentation of written rationale for selection of models, record of code verification (hand-calculation checks, comparison to other models), source of historical data, and source of new theory, calibration and sensitivity analyses results, and documentation of adjustments to parameter values due to calibration. Electronic data are backed up on individual computers, the PI's computer and on network server are backed up daily; data are

also backed up to an external hard drive weekly. In the event of a catastrophic systems failure, the tapes can be used to restore the data in less than one day's time. Data generated on the day of the failure may be lost, but can be reproduced from raw data in most cases.

Quarterly progress reports (QPRS) disseminated to the individuals listed in section A3 will note activities conducted in connection with BST and modeling, items or areas identified as potential problems, and any variations or supplements to the QAPP. Final reports on BST analysis, SELECT modeling analysis and the LDC analysis will be developed. Outcomes will be submitted to the established stakeholder group and utilized in future watershed planning development.

Corrective Action Reports (CARs) will be utilized when necessary (Appendix A). CARs will be maintained in an accessible location for reference at TWRI and will be disseminated to the individuals listed in section A3. CARs resulting in any changes or variations from the QAPP will be made known to pertinent project personnel and documented in updates or amendments to the QAPP.

All electronic data are backed up on an external hard drive monthly, compact disks weekly, and is simultaneously saved in an external network folder and the computer's hard drive. A blank CAR is presented in Appendix A and a blank COC form is presented in Appendix D. The TSSWCB may elect to take possession of records at the conclusion of the specified retention period.

### **QAPP Revision and Amendments**

Until the work described is completed, this QAPP shall be revised as necessary and reissued at least annually on the anniversary date, or revised and reissued within 60 days of significant changes, whichever is sooner. The last approved versions of QAPPs shall remain in effect until revised versions have been fully approved; the revision must be submitted to the TSSWCB for approval before the last approved version has expired. If the entire QAPP is current, valid, and accurately reflects the project goals and the organization's policy, the annual re-issuance may be done by a certification that the QAPP is current. This will be accomplished by editing the QAPP to update the revision number and date and then submitting a cover letter stating the status of the QAPP and a copy of new, signed approval pages for the QAPP.

QAPP amendments may be necessary to reflect changes in project organization, tasks, schedules, objectives and methods; address deficiencies and nonconformances; improve operational efficiency; and/or accommodate unique or unanticipated circumstances. Written requests for amendments that do not constitute a significant change are directed from the TWRI PM/QAO to the TSSWCB PM and are effective immediately upon approval by the TSSWCB PM and QAO. Amendments to the QAPP and the reasons for the changes will be documented and distributed to all individuals on the QAPP distribution list by the TWRI PM/QAO. Amendments shall be reviewed, approved, and incorporated into a revised QAPP during the annual revision process.

## Section B1: Sampling Process Design (Experimental Design)

### ***BST Analysis***

To provide sufficient water quality data to characterize bacteria loadings across the various flow regimes, NETMWD, under the *Assessment of Contact Recreation Use Impairments and Watershed Planning for Big Cypress Creek and Tributaries (Hart and Tankersley Creeks)* project (TSSWCB project 09-54) will conduct routine ambient monitoring once every 2 weeks at 14 stream sites (see Table A6.2) and 2 WWTFs for a total of 16 sampling sites. NETMWD will ensure that permission is obtained from both the TCEQ and the respective entities to monitor WWTFs at Mt. Pleasant and Pilgrim's Pride. This data will provide information that will allow for an estimate of possible contributions from wastewater discharges. NETMWD will conduct biased-flow monitoring under high flow (storm event) conditions at the same 14 stream sites and 2 WWTFs during at least 12 storm events. Field data and samples will be collected following procedures detailed in the *TCEQ SWQM Procedures, Volume 1 (RG-415)*.

Samples collected by NETMWD will be delivered to Ana-Lab for processing and analysis; Ana-Lab will provide a subset of collected water samples to SAML for BST (Table B1.1). SAML will perform Bacteroidales PCR on approximately 250 individual water samples collected by NETMWD between August 2009 and July 2010. The samples will include: 1) 12 sample events for each of the 14 stream sites; 2) 9 sample events for each of the 2 WWTFs; and 3) 4 sample events for each of the 14 stream sites and 2 WWTFs during storm events. SAML will also isolate and fingerprint (ERIC-RP) *E. coli* (one per site per sample event) from each of the 14 stream sites and 2 WWTFs for 4 sample events and also 2 storm events; this results in a total of 100 individual samples analyzed using ERIC-PCR.

**Table B1.1. Samples to be Analyzed using *Bacteroidales* PCR and ERIC-RP<sup>3</sup>**

'X' denotes a single sampling event <sup>1</sup>		2009				2010								Total # Samples
Parameter		Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	
<i>Bacteroidales</i>														
250 Samples	Stream (14)	X	X	X	X	X	X	X	X	X	X	X	X	168
	WWTFs (2)	X	X	X		X		X	X	X	X		X	18
	Storm - Stream (14) <sup>2</sup>	X			X			X			X			56
	Storm - WWTFs (2)	X			X			X			X			8
<i>E. coli</i> (ERIC-RP)														
100 Samples	Stream (14)	X			X			X			X			56
	WWTFs (2)	X	X		X			X	X		X			12
	Storm - Stream (14) <sup>2</sup>				X						X			28
	Storm - WWTFs (2)				X						X			4

<sup>1</sup> An 'X' denotes one complete subset (1 sample collected from each site) of samples collected to be analyzed for respective BST analysis.

<sup>2</sup> Approximately one storm event sample will be analyzed per site per quarter using *Bacteroidales* PCR and every other quarter using ERIC-RP.

<sup>3</sup> Sampling schedule may be adjusted depending upon the timing of rainfall events, run-off producing storms, and the need for any repeated measurements due to technical issues.

**Modeling Analysis**

Not relevant.

## Section B2: Sampling Method Requirements

### BST Analysis

NETMWD will conduct water quality monitoring in the study area through TSSWCB project 09-54. Samples will only be taken if water is flowing. If a site is not flowing but pooled or dry, that will be noted on the field data sheet. Water samples will be collected directly from the stream (midway in the stream channel) into containers as specified in Table B2.1. The sample container will be held upstream of the sampler and care will be taken to avoid contact with sediment and the surface micro layer of water. All samples will be transported in an iced container to Ana-Lab for analysis. A subset of water samples will be processed and shipped to SAML along with appropriate COC forms (Appendix C).

**Table B2.1. Container, Preservation, Temp., Sample Size, and Holding Time Requirements**

Parameter	Matrix	Container	Preservation	Temp.	Sample Size	Holding Time
<i>E. coli</i>	water	IDEXX bottle	Ice, dark	4°C	100 ml	8 hours <sup>1</sup>
<i>E. coli</i> water isolates	Modified m-TEC agar	Petri dish 50mm x 9mm	Ice/refrigeration	4°C	100 ml	24-48 hrs, then shipped to SAML on ice
<i>Bacteroidales</i>	Supor filters	7 oz Whirl-Pak bag	GITC buffer	-20 or -80°C	100 ml	6 hours <sup>1</sup> , filters indefinitely

<sup>1</sup> 8 hours to deliver to laboratory. In the case that this 8-hour holding time is not met, the *E. coli* quantitative count will be flagged and not reported, though the *Bacteroidales* PCR will still be valid.

### Recording Data

For the purposes of this section and subsequent sections, all field and laboratory personnel will: (1) write legibly in indelible, waterproof ink with no modifications, write-overs or cross-outs; (2) correct errors with a single line followed by an initial and date; and (3) close-out incomplete pages with an initialed and dated diagonal line.

### Deviations from Sampling Method Requirements or Sample Design, and Corrective Action

Examples of deviations from sampling method requirements include inadequate sample volume collected, failure to preserve samples appropriately, contamination of sample bottle during collection, storage temperature and holding time exceedance, and sampling at the wrong site. Deviations invalidate resulting data and may require corrective action including samples being discarded and re-collected. It is the responsibility of the SAML Project Leader and TWRI PM/QAO to ensure that the actions and resolutions to the problems are documented and that records are maintained in accordance with this QAPP. In addition, these actions and resolutions will be conveyed to the TSSWCB PM both verbally and in writing in QPRs and by completion of a CAR as shown in Appendix A. CARs will be included with QPRs. In addition, significant conditions (i.e., situations which, if uncorrected, could have a

serious effect on safety or on the validity or integrity of data) will be reported to the TSSWCB immediately both verbally and in writing.

**Modeling Analysis**

Not relevant.

## **Section B3: Sample Handling and Custody Requirements**

### **BST Analysis**

#### **Chain of Custody**

Proper sample handling and custody procedures ensure the custody and integrity of samples beginning at the time of sampling and continuing through transport, sample receipt, preparation, and analysis. The COC form is used to document sample handling during transfer from the field to the laboratory. The sample number, location, date, changes in possession and other pertinent data will be recorded in indelible ink on the COC. The sample collector (NETMWD) will sign the COC and transport it with the sample to the laboratory (Ana-Lab). At the laboratory, samples are inventoried against the accompanying COC. Any discrepancies will be noted at that time and the COC will be signed for acceptance of custody. Ana-Lab will conduct preliminary sample preparations and will store prepared samples prior to delivery of samples accompanied by a COC to SAML. Once received at SAML, samples are inventoried against the accompanying COC. Any discrepancies will be noted at that time and the COC will be signed for acceptance of custody. A blank COC form used on this project is included as Appendix C.

#### **Sample Labeling**

Samples will be labeled on the container with an indelible, waterproof marker. Label information will include site identification, date, sampler's initials, and time of sampling. The COC form will accompany all sets of sample containers.

#### **Sample Handling**

Following collection, samples will be placed on ice in an insulated cooler for transport to the laboratory (Ana-Lab). At the laboratory, samples will be placed in a refrigerated cooler dedicated to sample storage. The Laboratory Director has the responsibility to ensure that holding times are met with water samples. The holding time is documented on the COC. Following sample preparation, plates containing *E. coli* cultures will be stored at 4°C in a refrigerator for a maximum of 24-48 hours before shipment to SAML. Following *Bacteroidales* sample preparation, filters will be stored at Ana-Lab in a -20°C manual defrost freezer or an ultra-low (-80°C) freezer until delivery to SAML is arranged. Cultured *E. coli* samples will be delivered, overnight, from Ana-Lab to SAML in a cooler box with appropriate refrigerant methods to maintain appropriate temperatures; *E. coli* isolates will be shipped on blue ice or freezer blocks and *Bacteroidales* samples will be shipped on dry ice. Any problem will be documented with a CAR.

Specific shipping and handling methods are clearly outlined in Appendix C.

#### **Failures in Chain of Custody and Corrective Action**

All failures associated with COC procedures as described in this QAPP are immediately reported to the TWRI PM/QAO. These include such items as delays in transfer, resulting in holding time violations; violations of sample preservation requirements; incomplete

documentation, including signatures; possible tampering of samples; broken or spilled samples, etc. The TWRI PM/QAO will determine if the procedural violation may have compromised the validity of the resulting data. Any failures that have reasonable potential to compromise data validity will invalidate data and the sampling event should be repeated. The resolution of the situation will be reported to the TSSWCB PM in the QPR. CARs will be prepared by the TWRI PM/QAO and submitted to the TSSWCB PM along with QPRs.

**Modeling Analysis**

Not relevant.

## Section B4: Analytical Methods

### BST Analysis

The analytical methods are listed in Table B4.1 and described in detail in Appendix B.

*E. coli* in water samples will be quantified and isolated by Ana-Lab personnel using modified mTEC agar, EPA Method 1603 [EPA/821/R-02/023. September 2002. *Escherichia coli* in Water by Membrane Filtration Using Modified Membrane-Thermotolerant *Escherichia coli* (modified m-TEC) Agar]. The modified medium contains the chromogen 5-bromo-6-chloro-3-indolyl- $\beta$ -D-glucuronide (Magenta Gluc), which is catabolized to glucuronic acid (a red/magenta-colored compound) by *E. coli* that produces the enzyme  $\beta$ -D-glucuronidase. This enzyme is the same enzyme tested for using other substrates such as the fluorogenic reaction with 4-methylumbelliferyl- $\beta$ -D-glucuronide (MUG) observed by ultraviolet light (UV) fluorescence. *E. coli* colonies from the modified mTEC medium will be picked by SAML and streaked for purity on nutrient agar with MUG (NA-MUG) to confirm glucuronidase activity and culture purity. Cultures of selected isolates will be archived using glycerol freezing medium (-80°C). Inoculated plates will be incubated at 35±0.5°C for 2 hours to resuscitate stressed bacteria, then incubated at 44.5±0.2°C for approximately 20 to 24 hours. *E. coli* isolates obtained from ambient water samples from across the study area will be characterized using ERIC-PCR and RiboPrinting using AgriLife El Paso SOPs. DNA patterns of those isolates will be compared to the Texas Known Source Library of *E. coli* isolates from known animal and human sources collected throughout Texas. Water isolates will be identified to cattle, other livestock, avian and non-avian wildlife, domestic sewage, and pet sources (six-way split), as well as a broader three-way split of livestock, domestic sewage and wildlife.

**Table B4.1. Laboratory Analytical Methods**

Parameter	Method	Equipment Used
<i>E. coli</i>	EPA 1603	Filtration apparatus, incubator
<i>E. coli</i> RiboPrint fingerprint	AgriLife El Paso SOP	RiboPrinter
<i>E. coli</i> ERIC-PCR fingerprint	AgriLife El Paso SOP	PCR thermal cycler, gel electrophoresis apparatus
<i>Bacteroidales</i> PCR	AgriLife El Paso SOP	PCR thermal cycler, gel electrophoresis apparatus

EPA = Methods for Chemical Analysis of Water and Wastes, March 1983

SOP = Standard Operating Procedure

As outlined in Appendix B, 100 ml water samples will be collected and filtered for analysis of *Bacteroidales*. *Bacteroidales* DNA will be extracted from the filters and analyzed using PCR using El Paso AgriLife Research and Extension Center (EP AREC) SOPs.

All laboratory sampling areas and equipment will be sterilized with at least one or in any combination of the following methods: ethyl alcohol, bleach, UV light, or autoclave. All disposables will be placed in a heat-resistant biohazard bag and autoclaved prior to disposal.

### **Failures in Measurement Systems and Corrective Actions**

Failures in field and laboratory measurement systems involve, but are not limited to such things as instrument malfunctions, failures in calibration, blank contamination, QC samples outside QAPP defined limits, etc. In many cases, the field technician or lab analyst will be able to correct the problem. If the problem is resolvable by the field technician or lab analyst, then they will document the problem on the field data sheet or laboratory record and complete the analysis. If the problem is not resolvable, then it is conveyed to the SAML Director, who will make the determination in coordination with the TWRI PM/QAO. If the analytical system failure may compromise the sample results, the resulting data will not be reported to the TSSWCB as part of this project. The nature and disposition of the problem is reported on the data report. The TWRI PM/QAO will include this information in the CAR and submit with the QPR which is sent to the TSSWCB PM.

### **Modeling Analysis**

Not relevant.

## **Section B5: Quality Control Requirements**

### **BST Analysis**

Table A7.1 lists the required accuracy, precision, and completeness limits for the parameters of interest. It is the responsibility of the SCSC Project Leader to verify that the data are representative. The SCSC Project Leader also has the responsibility of determining that the 90 percent completeness criteria is met, or will justify acceptance of a lesser percentage. All incidents requiring corrective action will be documented through use of CARs (Appendix A). Laboratory audits, sampling site audits, and QA of field sampling methods will be conducted by the TSSWCB QAO or their designee.

### **Laboratory Blanks**

Ana-Lab will be conducting initial sample preparation including filtering, storage and shipping. As a result, evaluating laboratory blanks will be the primary responsibility of Ana-Lab. Laboratory blanks, or negative controls, consist of 100-ml aliquots of sterile distilled water that are processed in the same manner as a field sample, at the beginning and the end of a sample set. They are used to assess the sterilization techniques employed throughout the sample process. Laboratory blanks will be included at the beginning and the end of the sample set for each sampling event.

As a means to verify the correctness of the method and no cross contamination, SAML will also conduct lab blanks. The analysis of laboratory blanks should yield a value of no colonies detected. For *Bacteroidales* PCR, a laboratory blank will be analyzed with each batch of samples to ensure no cross-contamination occurs during sample processing. In addition, negative controls will be analyzed for each batch of PCR samples.

### **Positive Control**

Positive controls (a well-characterized *E. coli* strain or microbial community DNA from known fecal sources) will be analyzed by SAML for each batch of *E. coli* ERIC-PCR and RiboPrinting, and *Bacteroidales* PCR samples.

### **Laboratory Duplicate**

Laboratory duplicates are used to assess precision. A laboratory duplicate is prepared by splitting aliquots of a single sample (or a matrix spike or a laboratory control standard) in the laboratory. Both samples are carried through the entire preparation and analytical process. Laboratory duplicates are run at a rate of one per batch. Acceptability criteria are outlined in Table A7.1 of Section A7.

Precision is calculated by the relative percent difference (RPD) of duplicate results as defined by 100 times the difference (range) of each duplicate set, divided by the average value (mean) of the set. For duplicate results,  $X_1$  and  $X_2$ , the RPD is calculated from the following equation:

$$\text{RPD} = \frac{(\mathbf{X_1 - X_2}) \times 100}{(\mathbf{X_1 + X_2}) \div 2}$$

A bacteriological duplicate is considered to be a special type of laboratory duplicate and applies when bacteriological samples are run in the field as well as in the laboratory. Bacteriological duplicate analyses are performed on samples from the sample bottle on a 10% basis. Results of bacteriological duplicates are evaluated by calculating the logarithm of each result and determining the range of each pair.

Performance limits and control charts are used to determine the acceptability of duplicate analyses. Precision limits for bacteriological analyses are defined in Table A7.1 and applies to samples with concentrations >10 cfu/100 ml.

#### **Failures in Quality Control and Corrective Action**

Notations of blank contamination will be noted in QPRs and the final report. Corrective action will involve identification of the possible cause (where possible) of the contamination failure. Any failure that has potential to compromise data validity will invalidate data, and the sampling event should be repeated. The resolution of the situation will be reported to the TSSWCB in the QPR. The CARs will be maintained by the SCSC Project Leader and the TSSWCB PM.

#### **Modeling Analysis**

Not relevant.

## Section B6: Equipment Testing, Inspection, & Maintenance Requirements

### **BST Analysis**

To minimize downtime of all measurement systems, spare parts for laboratory equipment will be kept in the laboratory, and all laboratory equipment must be maintained in a working condition. All laboratory equipment will be tested, maintained, and inspected in accordance with manufacturer's instructions and recommendation in *Standard Methods for the Examination of Water and Wastewater, 21<sup>st</sup> Edition*. Maintenance and inspection logs will be kept on each piece of laboratory equipment.

All field equipment used to collect samples for this BST analysis will be tested, inspected and maintained in accordance with the TSSWCB QAPP 09-54.

Records of all tests, inspections, and maintenance will be maintained and log sheets kept showing time, date, and analyst signature. These records will be available for inspection by the TSSWCB.

Failures in any testing, inspections, or calibration of equipment will result in a CAR and resolution of the situation will be reported to the TSSWCB in the QPR. The CARs will be maintained by the SCSC Project Leader and the TSSWCB PM.

**Table B6.1. Equipment Inspection and Maintenance Requirements**

<b>Equipment</b>	<b>Relevant Testing, Inspection &amp; Maintenance Requirement</b>
Thermometers	Per manufacturer & annual preventative maintenance
PCR Thermal cycler	Per manufacturer & annual preventative maintenance
RiboPrinter	Per manufacturer & annual preventative maintenance
Water deionization units	Per manufacturer & annual preventative maintenance
Media dispensing apparatus	Per manufacturer & annual preventative maintenance
Autoclaves	Per manufacturer & annual preventative maintenance
Refrigerator	Per manufacturer & annual preventative maintenance
Ultra Low Freezer	Per manufacturer & annual preventative maintenance
Membrane filter equipment	Per manufacturer & annual preventative maintenance
Ultraviolet sterilization lamps	Per manufacturer & annual preventative maintenance
Biological safety cabinet	Per manufacturer & annual preventative maintenance
Incubators	Per manufacturer & annual preventative maintenance
Glassware and plastic ware	Per manufacturer & annual preventative maintenance
Utensils and containers	Per manufacturer & annual preventative maintenance
Dilution water bottles	Per manufacturer & annual preventative maintenance

### **Modeling Analysis**

Not relevant.

## **Section B7: Instrument Calibration and Frequency**

### **BST Analysis**

All instruments or devices used in obtaining environmental data will be calibrated prior to use. Each instrument has a specialized procedure for calibration and a specific type of standard used to verify calibration. The instruments requiring calibration are listed below in Table B7.1.

All calibration procedures will meet the requirements specified in the approved methods of analysis. The frequency of calibration as well as specific instructions applicable to the analytical methods recommended by the equipment manufacturer will be followed. All information concerning calibration will be recorded in a calibration logbook by the person performing the calibration and will be accessible for verification during a laboratory audit.

All instruments or devices used in obtaining environmental data will be used according to appropriate laboratory practices. Written copies of SOPs are available for review upon request.

Standards used for instrument or method calibrations shall be of known purity and be NIST traceable whenever possible. When NIST traceability is not available, standards shall be of American Chemical Society or reagent grade quality, or of the best attainable grade. All certified standards will be maintained traceable with certificates on file in the laboratory. Dilutions from all standards will be recorded in the standards log book and given unique identification numbers. The date, analyst initials, stock sources with lot number and manufacturer, and how dilutions were prepared will also be recorded in the standards log book.

Failures in any testing, inspections, or calibration of equipment will result in a CAR and resolution of the situation will be reported to the TSSWCB in the QPR. The CARs will be maintained by the SCSC Project Leader and the TSSWCB PM.

**Table B7.1. Instrument Calibration Requirements**

<b>Equipment</b>	<b>Relevant Calibration Requirement</b>
RiboPrinter	Per manufacturer & annual preventative maintenance
PCR Thermal Cycler	Per manufacturer & annual preventative maintenance

### **Modeling Analysis**

Not relevant.

## **Section B8: Inspection/Acceptance Requirements for Supplies and Consumables**

### **BST Analysis**

All standards, reagents, media, plates, filters, and other consumable supplies are purchased from manufacturers with performance guarantees, and are inspected upon receipt for damage, missing parts, expiration date, and storage and handling requirements. Labels on reagents, chemicals, and standards are examined to ensure they are of appropriate quality, initialed by staff member and marked with receipt date. Volumetric glassware is inspected to ensure class "A" classification, where required. Media will be checked as described in QC procedures. All supplies will be stored as per manufacturer labeling and discarded past expiration date. In general, supplies for microbiological analysis are received pre-sterilized, used as received, and not re-used.

### **Modeling Analysis**

Not relevant.

## Section B9: Data Acquisition Requirements (Non-direct Measurements)

### **BST Analysis**

Historical data will be retrieved from the TCEQ SWQM Information System (SWQMIS). Historical data were collected and analyzed consistently with TCEQ *SWQM Procedures* under the SWQM QAPP or Clean Rivers Program QAPP and therefore are considered representative of ambient conditions and will be comparable to data collected under this project. Table B9.1 shows the date range of data for each of six existing sites for which SWQMIS has historical data. The mean and median will be computed for each parameter as well as the number of water quality criteria exceedances, as applicable. This information will be compared statistically to the results of data collected under this project. Due to the historical data's comparability to the data collected under this project, there are not limitations on their use.

**Table B9.1 Historical Data**

Site Number	Site Name	Date Range of Historical Data
10261	Tankersley Creek at FM 3417	12/00 – 04/05
10263	Tankersley Creek at FM 127	05/04 – 04/05
10264	Tankersley Creek at FM 889	10/02 – 04/05
10266	Hart Creek at Titus CR SE-12	10/02 – 08/07
10272	Hart Creek at SH 49	10/02 – 08/03
10308	Big Cypress Creek at SH 11	12/00 – 08/07
10310	Big Cypress Creek at US 271	5/07 – 08/07

Additionally, data collected by NETMWD under the *Assessment of Contact Recreation Use Impairments and Watershed Planning for Big Cypress Creek and Tributaries (Hart and Tankersley Creeks)* project (TSSWCB Project 09-54) will be collected in accordance with that approved QAPP. Data utilized from this project will include water quality samples collected from designated stream crossings and WWTFs and will be delivered to Ana-Lab for processing within required holding times. Prepared samples will be stored and shipped to SAML.

### **Modeling Analysis**

The NETMWD is a partner in the Clean Rivers Program for the state of Texas. As such, they collect data on a regular basis for routine water quality assessment as part of the state's mandate for Clean Water Act (CWA) §305(b) – Water Quality Inventory Report. These data also are used by Texas for consideration of water bodies to be added to their list of impaired water body segments, as described in CWA §303(d). Additional data obtained from the TCEQ are from the SWQMIS database.

All data used in the modeling procedures for this project are collected in accordance with approved quality assurance measures under the state's Clean Rivers Program, TCEQ, Texas Water Development Board, USDA, National Weather Service, or USGS. Future data collection carried out by NETMWD and supported by TSSWCB's TMDL Program funding (TSSWCB Project 09-54) will be incorporated into the modeling process as the data become available. Those data will be collected under a separate QAPP for TSSWCB Project 09-54.

GIS data to be used are 2004 and 2005 NAIP (National Agricultural Imagery Program) aerial photos, SSURGO (Soil Survey Geographic) and CBMS (Computer Based Mapping System) soils, USGS NLCD (National Land Cover Dataset) landuse, National Hydrography Dataset (NHD), Census data (2000), Agricultural Census data from USDA-NASS (2002), and the USGS 30-meter resolution digital elevation model (DEM). Depending on the availability of the GIS layers from different data sources, efforts will be made to update the spatial data to the most recent year.

Because most historical data is of known and acceptable quality and were collected and analyzed in a manner comparable and consistent with needs for this project, no limitations will be placed on their use, except where known deviations have occurred.

## **Section B10: Data Management**

### **BST Analysis**

#### **Field Collection and Management of Routine Samples**

All field collection to be completed by NETMWD as outlined in the TSSWCB QAPP 09-54. A field data sheet is filled out in the field for each site visit. Samples collected will be labeled and placed in an iced, insulated chest for transportation to the laboratory. A blank COC form is presented in Appendix C. Site name, time of collection, comments, and other pertinent data are copied from the field data sheet to the COC.

#### **Laboratory Data**

Once the samples are received at Ana-Lab, samples are logged and stored at 4°C until processed. The COC will be checked for number of samples, proper and exact ID number, signatures, dates, and type of analysis specified. NETMWD will be notified if any discrepancy is found and proper corrections made. The COC and accompanying sample bottles are submitted to the Ana-Lab analyst, with relinquishing and receiving personnel both signing and dating the COC. Processed samples will be stored at Ana-Lab in a refrigerator or freezer (depending upon sample type) until shipment of samples to SAML is arranged. Samples will be transported with COC, with relinquishing and receiving personnel both signing and dating the COC. All COC and bacteriological data will be manually entered into an electronic spreadsheet. The electronic spreadsheet will be created in Microsoft® Excel software on an IBM-compatible microcomputer with a Windows® operating system. The project spreadsheet will be maintained on the computer's hard drive, which is also simultaneously saved in a network folder. Data manually entered in the database will be reviewed for accuracy by the SCSC Project Lead or TWRI PM/QAO to ensure that there are no transcription errors. Hard copies of data will be printed and housed in the laboratory for a period of five years. Any COCs and bacteriological records related to QA/QC of bacteriological procedures will be housed at the SAML. All pertinent data files will be backed up monthly on an external hard drive. Current data files will be backed up on an external hard drive monthly and stored in separate area away from the computer. Original data recorded on paper files will be stored for at least five years. Electronic data files will be archived to CD after approximately the end of the project, and then stored with the paper files for the remaining 4 years.

#### **Data Validation**

Following review of laboratory data, any data that is not representative of environmental conditions, because it was generated through poor field or laboratory practices, will not be submitted to the TSSWCB. This determination will be made by the SCSC Project Leader, TWRI PM/QAO, TSSWCB QAO, and other personnel having direct experience with the data collection effort. This coordination is essential for the identification of valid data and the proper evaluation of that data. The validation will include the checks specified in Table D2.1.

### **Data Dissemination**

At the conclusion of the project, the SCSC Project Leader will provide a copy of the complete project electronic spreadsheet via recordable CD media to the TSSWCB PM, along with the final report. The TSSWCB may elect to take possession of all project records. However, summaries of the data will be presented in the final project report.

### **Modeling Analysis**

#### **Systems Design**

BAEN uses laptop personal computers and desktop personal computers. The computers run Windows XP or Vista operating system. Softwares include Microsoft® Word, Microsoft® Excel, Microsoft® Access, and a Statistical Analysis System database management system run through Windows XP operating system. All GIS analysis will be performed using ArcGIS 9x.

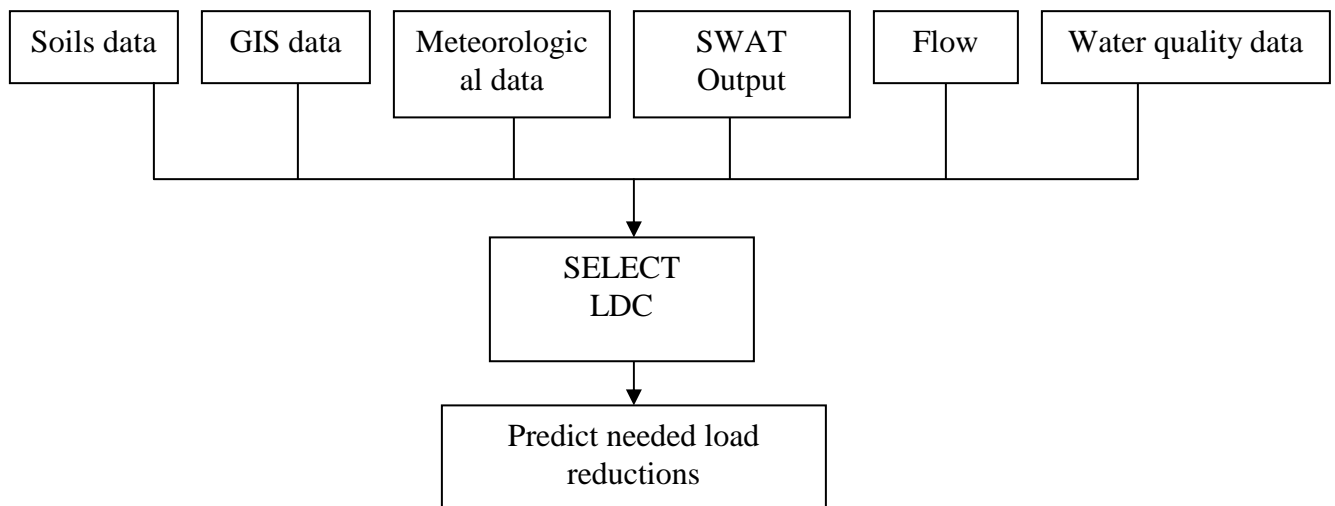
#### **Backup and Disaster Recovery**

The personal computer drives are backed up on a weekly basis to a tape drive and on a monthly basis to an external hard drive for storage in a secure secondary location. In the event of a catastrophic systems failure, the tapes can be used to restore the data in less than one day's time. Data generated on the day of the failure may be lost, but can be reproduced from raw data in most cases.

#### **Archives and Data Retention**

Original data recorded on paper files are stored for at least five years. Data in electronic format are stored on tape drives in a climate controlled, fire-resistant storage area on either the Texas A&M University campus.

**Figure B10-1. Information Dissemination Diagram**



## Section C1: Assessments and Response Actions

### **BST Analysis**

The following table presents types of assessments and response actions for data collection activities applicable to the QAPP.

**Table C1.1 Assessments and Response Actions (SAML)**

Assessment Activity	Approximate Schedule	Responsible Party	Scope	Response Requirements
Status Monitoring Oversight	Continuous	TWRI Project Manager	Monitoring of project status and records to ensure requirements are being fulfilled. Monitoring and review of laboratory performance and data quality	Report to TSSWCB in QPR. Ensure project requirements are being fulfilled.
Laboratory Inspections	At least one per life of the project	TSSWCB QAO	Analytical and QC procedures employed at laboratory	30 days to respond in writing to TSSWCB to address corrective actions

### **Corrective Action**

The TWRI PM/QAO and SAML Project Leader are responsible for implementing and tracking corrective action as a result of audit findings. Records of audit findings and corrective actions are maintained by the TSSWCB PM and TWRI PM/QAO. Corrective action documentation will be submitted to the TSSWCB PM with the QPR. If audit findings and corrective actions cannot be resolved, then the authority and responsibility for terminating work is specified in agreements or contracts between participating organizations.

### **Modeling Analysis**

Table C1.2 presents the types of assessments and response actions for activities applicable to the QAPP.

In addition to those listed in Table C1.2, the following assessment and response actions will be applied to modeling activities. As described in Section B9 (Non-direct Measurements), modeling staff will evaluate data to be used in calibration and as model input according to criteria discussed in Section A7 (Quality Objectives and Criteria for Model Inputs/Outputs Data) and will follow-up with the various data sources on any concerns that may arise.

**Table C1.2 Assessments and Response Actions (BAEN)**

Assessment Activity	Approximate Schedule	Responsible Party(ies)	Scope	Response Requirements
Status Monitoring Oversight, etc.	Continuous	TWRI, BAEN	Monitoring of the project status and records to ensure requirements are being fulfilled. Monitoring and review of performance and data quality.	Report to project lead in Quarterly Report
Technical Systems Audit	Minimum of one during the course of this project.	TSSWCB QAO	The assessment will be tailored in accordance with objectives needed to assure compliance with the QAPP. Facility review and data management as they relate to the project.	30 days to respond in writing to the TSSWCB QAO to address corrective actions

The model calibration procedure is discussed in Section D2 (Validation and Verification Methods), and criteria for acceptable outcomes are provided in Section A7 (Quality Objectives and Criteria for Model Inputs/Outputs).

Results will be reported to the project QAO in the format provided in Section A9. If agreement is not achieved between the calibration standards and the predictive values, corrective action will be taken by the Project Manager to assure that the correct files are read appropriately and the test is repeated to document compliance. Corrective action is required to ensure that conditions adverse to quality data are identified promptly and corrected as soon as possible. Corrective actions include identification of root causes of problems and successful correction of identified problem. CARs (Appendix A) will be filled out to document the problems and the remedial action taken. Copies of CARs will be included with the TWRI's annual Quality Assurance report. The Quality Assurance report will discuss any problems encountered and solutions made. These QA reports are the responsibility of the TWRI PM/QAO and will be disseminated to individuals listed in section A3. If the predicted value cannot be brought within calibration standards, the QAO will work with TSSWCB to arrive at an agreeable compromise.

Software requirements, software design, or code are examined to detect faults, programming errors, violations of development standards, or other problems. All errors found are recorded at the time of inspection, with later verification that all errors found have been successfully corrected. Software used to compute model predictions are tested to assess its performance relative to specific response times, computer processing usage, run time, convergence to solution, stability of the solution algorithms, the absence of terminal failures, and other quantitative aspects of computer operation.

Checks are made to ensure that the computer code for each module is computing module outputs accurately and within any specific time constraints. The full model framework is tested as the ultimate level of integration testing to verify that all project-specific requirements have been implemented as intended. All testing performed on the original version of the module or linked modules is repeated to detect new "bugs" introduced by changes made in the code to correct a model.

## **Section C2: Reports to Management**

QPRs will be generated by TWRI and will note activities conducted in connection with BST work, water quality modeling, items or areas identified as potential problems, and any variation or supplement to the QAPP. CARs will be utilized when necessary (Appendix A) and will be maintained in an accessible location for reference at TWRI. The CARs that result in changes or variations from the QAPP will be made known to pertinent project personnel, documented in an update or amendment to the QAPP and distributed to personnel listed in Section A3. Following any audit performed by TWRI, a report of findings, recommendations and responses are sent to the TSSWCB PM in the QPR.

Laboratory data reports contain the results of all analyses, as well as specified QC measures listed in section B5. This information is reviewed by the TWRI PM/QAO and compared to the pre-specified acceptance criteria to determine acceptability of data. This information is available for inspection by the TSSWCB.

If the procedures and guidelines established in this QAPP are not successful, corrective action is required to ensure that conditions adverse to quality data are identified promptly and corrected as soon as possible. Corrective actions include identification of root causes of problems and successful correction of identified problem. CARs will be filled out to document the problems and the remedial action taken. Copies of CARs will be included with the quarterly project report. The CARs will discuss problems encountered and solutions made. These reports will be disseminated to individuals listed in section A3.

The final report for this project will be individual technical reports detailing the results of BST and water quality modeling work conducted under this QAPP. Items in these reports will include a very brief description of methodologies utilized, a detailed narrative regarding specific BST/modeling findings and a discussion/conclusions section that highlights the implications of the BST/modeling findings.

## **Section D1: Data Review, Validation and Verification**

### **BST Analysis**

All data obtained from field and laboratory measurements will be reviewed and verified for conformance to project requirements, and then validated against the DQOs which are listed in Section A7. Only those data which are supported by appropriate QC data and meet the DQOs defined for this project will be considered acceptable. This data will be submitted to the TSSWCB.

The procedures for verification and validation of data used in BST analysis are described in Section D2. The SAML Director is responsible for ensuring that laboratory data are scientifically valid, defensible, of acceptable precision and accuracy, and reviewed for integrity. The TWRI PM/QAO will be responsible for ensuring that all data are properly reviewed and verified, validated, and submitted in the required format as described by the TSSWCB PM. Finally, the TWRI PM/QAO is responsible for validating that all data to be reported meet the objectives of the project and are suitable for reporting to TSSWCB.

### **Modeling Analysis**

The procedures for verification and validation of data used in water quality modeling analysis are described in Section D2, below. The BAEN Project Co-Leader is responsible for ensuring that data are properly reviewed, verified, and submitted in the required format for the project database. Finally, the TWRI PM/QAO is responsible for validating that all data collected meet the DQOs of the project and are suitable for reporting.

## **Section D2: Validation Methods**

### **BST Analysis**

All field and laboratory data will be reviewed, verified and validated to ensure they conform to project specifications and meet the conditions of end use as described in Section A7. The staff and management of the respective field, laboratory, and data management tasks are responsible for the integrity, validation and verification of the data each task generates or handles throughout each process. The field and laboratory tasks ensure the verification of raw data, electronically generated data, and data on COC forms and hard copy output from instruments.

Verification, validation and integrity review of data will be performed using self-assessments and peer review, as appropriate to the project task, followed by technical review by the manager of the task. The data to be verified (listed by task in Table D2.1) are evaluated against project specifications (Section A7) and are checked for errors, especially errors in transcription, calculations, and data input. Potential outliers are identified by examination for unreasonable data. If a question arises or an error or potential outlier is identified, the manager of the task responsible for generating the data is contacted to resolve the issue. Issues which can be corrected are corrected and documented electronically or by initialing and dating the associated paperwork. If an issue cannot be corrected, the task manager consults with the TWRI PM/QAO to establish the appropriate course of action, or the data associated with the issue are rejected.

The SCSC Project Lead, with assistance from the TWRI PM/QAO, is responsible for validating that the verified data are scientifically valid, legally defensible, of known precision, accuracy, integrity, meet the DQOs of the project, and are reportable to TSSWCB. One element of the validation process involves evaluating the data for anomalies. The SCSC Project Lead may designate other experienced water quality experts (BRA Environmental Planner or other designated employee) familiar with the waterbodies under investigation to perform this evaluation. Any suspected errors or anomalous data must be addressed by the manager of the task associated with the data, before data validation can be completed.

A second element of the validation process is consideration of any findings identified during the monitoring systems audit conducted by the TWRI PM/QAO or TSSWCB QAO. Any issues requiring corrective action must be addressed, and the potential impact of these issues on previously collected data will be assessed. Finally, the TWRI PM/QAO validates that the data meet the DQOs of the project and are suitable for reporting to the TSSWCB.

**Table D2.1. Data Verification Procedures**

<b>Data to be Verified</b>	<b>TWRI PM /QAO</b>	<b>SAML Director</b>	<b>Ana- Lab QAO</b>	<b>TSSWCB PM/QAO</b>
Analysis techniques consistent with SOPs and QAPP	X	X	X	X
Instrument calibration data complete		X	X	X
Bacteriological records complete		X	X	X
Sample documentation complete		X	X	X
Sample identifications		X	X	X
COC complete/acceptable		X	X	X
Sample preservation and handling		X	X	X
Holding times		X	X	X
QC samples analyzed at required frequencies		X	X	X
QC samples within acceptance limits		X	X	X
Instrument readings/printouts		X	X	X
Calculations		X		X
Laboratory data verification for integrity, precision, accuracy, and validation		X	X	X
Laboratory data reports		X	X	X
Data entered in required format		X	X	X
Site ID number assigned				X
Absence of transcription error		X	X	X
Reasonableness of data		X	X	X
Electronic submittal errors		X	X	X
Sampling and analytical data gaps		X	X	X

### **Modeling Analysis**

There is no validation and calibration for the SELECT model or LDC as they are data processors.

## **Section D3: Reconciliation with User Requirements**

### **BST Analysis**

Data produced by this project will be evaluated against the established DQOs and user requirements to determine if any reconciliation is needed. Reconciliation concerning the quality, quantity or usability of the data will be reconciled with the user during the data acceptance process. CARs will be initiated in cases where invalid or incorrect data have been detected. Data that have been reviewed, verified, and validated will be summarized for their ability to meet the DQOs of the project and the informational needs of water quality agency decision-makers and watershed stakeholders.

The final data for the project will be reviewed to ensure that it meets the requirements as described in this QAPP. Data summaries along with descriptions of any limitations on data use will be included in the final report. Only BST data that has met the DQOs described in this QAPP will be reported and included in the final project report. Data and information produced thru this project will provide needed information pertaining to watershed characteristics, potential sources of pollution and will aid in the selection of BMPs to address identified water quality issues. Ultimately, stakeholders will use the information produced by this project for the development of appropriate measures to address water quality concerns in the study area. Information produced by this project will be used (through TSSWCB project 09-54) for watershed decisions regarding the development of a use attainability analysis, a TMDL or a WPP.

### **Modeling Analysis**

The modeling framework developed for this project will be used to evaluate water quality issues in the Big Cypress Creek Watershed. It will provide information pertaining to watershed characteristics and to the prediction of possible pollution, the sources of this pollution and will provide critical information to assist in identifying management practices to prevent pollution loading in area streams. This, in turn, will be useful for later TMDL or WPP development.

The final data will be reviewed to ensure that it meets the requirements as described in this QAPP. CARs will be initiated in cases where invalid or incorrect data have been detected. Data that have been reviewed, verified, and validated will be summarized for their ability to meet the DQOs of the project and the informational needs of water quality agency decision-makers. These summaries, along with a description of any limitations on data use, will be included in the final report.

## References

- Cleland, B. 2003. TMDL Development from the “bottom up” – Part III: Duration Curves and wet-weather assessments. America’s Clean Water Foundation, Washington, DC.
- Stiles, T.C., 2001. A simple method to define bacteria TMDLs in Kansas. KS Dept. of Health and Environment. Topeka, KS. <http://www.wef.org/pdffiles/TMDL/Stiles.pdf> (last accessed, 9/12/2006).
- Zeckoski, R.W., B.L. Benham, S.B. Shan, M.L. Wolfe, K.M. Brannan, M. Al-Smadi, T.A. Dillaha, S. Mostaghimi, and C.D. Heatwole, 2005. BSLC: A tool for bacteria source characterization for watershed management. Transactions of ASAE, 21(5): 879-889.

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## **Appendix A: Corrective Action Report**

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## Corrective Action Report

**SOP-QA-001**

**CAR #:**\_\_\_\_\_

Date:\_\_\_\_\_

Area/Location:\_\_\_\_\_

Reported by:\_\_\_\_\_

Activity:\_\_\_\_\_

State the nature of the problem, nonconformance or out-of-control situation:

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---

Possible causes:

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---

---

Recommended Corrective Actions:

---

---

---

CAR routed to:\_\_\_\_\_

Received by:\_\_\_\_\_

Corrective Actions taken:

---

---

---

---

Has problem been corrected?:

YES

NO

Immediate Supervisor:\_\_\_\_\_

Program Manager:\_\_\_\_\_

TWRI Quality Assurance Officer:\_\_\_\_\_

TSSWCB      Quality      Assurance      Officer:\_\_\_\_\_

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## **APPENDIX B. STANDARD OPERATING PROCEDURES**

B-1: Archival of <i>Escherichia coli</i> Isolates	66
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## **B-1: Archival of *Escherichia coli* Isolates**

*Note: All handling of cultures will be performed using a Class 2 biological safety cabinet to minimize the exposure of laboratory personnel to pathogens. These isolates should be from colonies which have been plated for purity several times and lab personnel are confident purity has been achieved.*

1. Select a well-isolated colony of purified *E. coli*. (Examine the cultures using a long-wave handheld UV lamp, colonies will fluoresce).
2. Using a bacteriological loop, transfer the colony to a labeled sterile cryovial containing 1 mL of tryptone soy broth (TSB) with 20% reagent grade glycerol.
3. Firmly cap the cryovial and verify that the cells have been resuspended by vortexing for several seconds; then plunge into liquid nitrogen until frozen.
4. Immediately transfer to a cryostorage box and place in -70 to -80°C freezer. Cultures may be stored for several years under these conditions.
5. To recover cultures from frozen storage, remove the cultures from the freezer and place the cryovials in a freezer block. *Do not allow cultures to thaw.*
  - a. Using a bacteriological loop, scrape the topmost portion of the culture and transfer to growth medium, being careful not to contaminate the top or inside of the vial. Invert and incubate plates at 35 to 37°C for 20 to 24 h.
  - b. Reclose the cryovial before the contents thaw and return to the freezer.

Casarez, E. A.; Pillai, S. D.; Mott, J.; Vargas, M.; Dean, K.; Di Giovanni, G. D. (2007). "Direct comparison of four bacterial source tracking methods and a novel use of composite data sets." J. Appl. Microbiol. In press doi:10.1111/j.1365-2672.2006.03246.x.

## **B-2: ERIC-PCR of *Escherichia coli***

1. Select isolated colonies from overnight cultures of *E. coli* isolates on Brain-Heart Infusion (BHI) plates.
2. Transfer colonies using a 1 µL loop to a sterile microfuge tube containing 100 µL of sterile molecular grade water; vortex briefly to suspend cells.
3. Prepare sufficient PCR Master Mix for samples, including one blank per 10 samples to account for volume loss due to repeat pipetting. Prepare Master Mix for each sample as noted below. One full PCR batch on the MJ Research Cyclor 48 well-plate will have 46 samples, *E. coli* QC101, and a no template control.

### **ERIC-PCR Master Mix – 24 samples + 2 blanks, prepare X 2 for full 48-well plate**

<b>MASTER MIX</b>	<b>Amt (uL)</b>	<b>Final Calc</b>	<b>Final Units</b>
dH <sub>2</sub> O	<b>819</b>		
10X PCR buffer I w Mg	<b>130</b>	1	X (1.5 mM)
20 mM dNTP	<b>13</b>	200	uM each
ERIC Primer Mix	<b>130</b>	600	nM each
BSA (30 mg/ml)	<b>65</b>	1.5	ug/uL
AmpliTaqGold (Units)	<b>13</b>	2.5	Units/rxn

4. Dispense 45 µl of Master Mix for each sample into the appropriate well of PCR plate.
5. Briefly vortex cell suspensions, then add 5 µl of each cell suspension to the appropriate PCR well.
6. Carefully seal plate using an adhesive PCR cover.
7. Load the plate into the thermal cycler and run under the “ERIC-PCR” program with the following cycling conditions:
  - a. Initial denaturation at 95°C for 10 min
  - b. 35 Cycles:
    - i. Denaturation at 94°C for 30 sec
    - ii. Annealing at 52°C for 1 min
    - iii. Extension at 72°C for 5 min
  - c. Final Extension at 72°C for 10 min
8. Store completed reactions at -20°C until analyzed by gel electrophoresis.

9. Prepare a 250 mL, 2% agarose gel using a 500 mL bottle. Add 250 mL of 1 X Tris/Borate/EDTA (TBE) buffer and 5.0 g agarose. Microwave until agarose is fully dissolved, tighten cap and let cool 1 to 2 minutes, then pour agarose into casting tray with 30-tooth, 1 mm thick comb.
10. Allow gel to solidify for approximately 30 minutes on the bench, then without removing comb place in Ziploc bag and solidify overnight in the refrigerator. The next day carefully remove comb, transfer to gel tank containing pre-cooled 1X TBE buffer. Replace TBE in gel tank after it has been used twice.
11. The following items will be needed for electrophoresis:

100 bp ladder (0.33 µg/10 µL) (1500 µL final, enough for 150 lanes)

200 µL Roche DNA Marker XIV (Cat. #1721933) 0.25 µg/µL 100 bp ladder (add reagents below to a full tube of marker)

300 µL 6X ERIC-PCR loading buffer (see recipe below)

150 µL 10X PCR buffer

850 µL molecular grade water

Store in cold room

6X ERIC-PCR Loading Buffer

25 mg bromphenol blue (0.25%)

1.5 g ficoll 400 (15%)

Add molecular grade water to 10 mL, divide into 1 mL aliquots and freeze, the aliquot currently being used can be stored in the cold room

ERIC-PCR Blank

100 µL 10X PCR buffer

200 µL 6X ERIC-PCR loading buffer

900 µL molecular grade water

Store in cold room

Ethidium Bromide Stain (0.5 µg/mL)

1250 mL 1X TBE

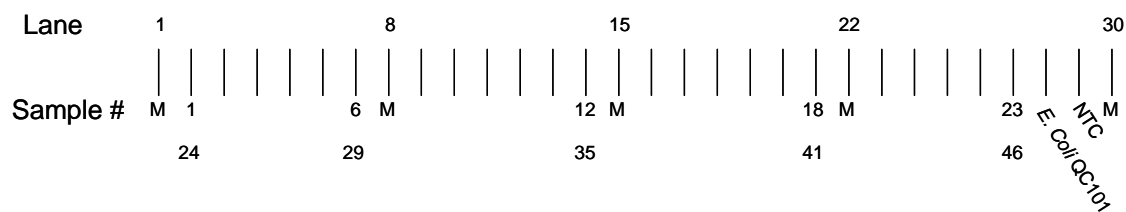
62.5 µL ethidium bromide (Sigma, 10 mg/mL)

Store covered at room temp, can use up to 5 times by adding 10 µL ethidium bromide each additional use

12. Mix 10 µL of 6X ERIC-PCR Loading Buffer to each PCR well and mix with pipette tip.

13. Load the gel in the cold room as follows (max. of 23 samples + QC101 + NTC per gel):

- a. Load 10 µl of 100 bp ladder (0.33 µg) into the first lane
- b. Load 10 µl of sample ERIC-PCR reactions into next 6 lanes
- c. Load 10 µl of 100 bp ladder (0.33 µg)
- d. Load 10 µl of sample ERIC-PCR reactions into next 6 lanes
- e. Load 10 µl of 100 bp ladder (0.33 µg)
- f. Load 10 µl of sample ERIC-PCR reactions into next 6 lanes
- g. Load 10 µl of 100 bp ladder (0.33 µg)
- h. Load 10 µl of sample ERIC-PCR reactions into next 5 lanes
- i. Load PCR Batch *E. coli* QC101 and NTC into next 2 lanes
- j. Load 10 µl of 100 bp ladder (0.33 µg)



If running a gel with fewer samples, follow steps above until last sample, followed by *E. coli* QC101, NTC and ladder, then load ERIC-PCR Blank into remaining lanes on gel.

14. Start electrophoresis power supply set at 100 volts, run for 1 hour.

15. Stop power supply, set time to "000", set voltage to 200 and start circulating pump at setting #2, run for 4 hours.
16. After electrophoresis, stain gel in Ethidium Bromide Stain for 20 minutes with agitation (save stain, see Step 13).
17. Destain gel for 10 minutes in 1X TBE buffer. Save destain, can be used 3 times then discard.
18. Follow Gel Imager SOP for image capture. Save digital photograph as a TIFF file (default) and print a hardcopy for notebook.

Casarez, E. A.; Pillai, S. D.; Mott, J.; Vargas, M.; Dean, K.; Di Giovanni, G. D. (2007). "Direct comparison of four bacterial source tracking methods and a novel use of composite data sets." J. Appl. Microbiol. In press doi:10.1111/j.1365-2672.2006.03246.x.

## **B-3: RiboPrinting of *Escherichia coli***

### **Storing and Handling Disposables**

Check the lot expiration date on each label for details and rotate the stock to optimize use.

#### Heating membrane and probe (MP) Base

After storage and the temperature changes that occur during shipment, the oxygen in the buffer loaded in the MP base may need to be removed before use. This is called degassing and is accomplished by heating the base pack overnight in your incubator.

To degas buffer:

1. Place enough MP base packs for the next day's production in their storage pouches in an incubator set at 37°C.
2. Allow the base pack to degas for 16 to 24 hours prior to loading in the characterization unit. You may do this while you are incubating samples, since the base packs are sealed in their pouches. This procedure allows you to start a batch immediately at the beginning of the next shift.
3. If you do not use the heated base packs, you can return them to storage and reuse them. These base packs should be heated again before reuse since temperature cycling affects oxygen content in the buffer.

#### Preparing Lysing Agent (for *Staphylococcus* and lactic-acid bacteria only)

Lysing agent (A and B) is shipped frozen and must be stored at -20°C. Lysing agent must be thawed before use. This only takes about 5 minutes. If the lysing agent will not be used again for more than 2 hours, the material should be returned to the freezer. Lysing agent can be re-frozen several times with no effect on performance.

## **Sample Preparation Procedures**

### **1. Incubate and Inspect the Samples**

Use BHI agar plates prepared within the last 30 days. Do not use plates that appear dry or dehydrated. Such plates can cause problems when you attempt to "pick" the colonies for use in the RiboPrinter® system.

1. Using a pure isolated colony as the source, streak BHI agar plates heavily in the upper portion of the plate to create a lawn. Streak the remainder of the plate lightly to create single colonies.
2. Follow standard laboratory techniques. Heat plates for 18 to 30 hours in a humidified incubator at 37 °C.

### **2. Transfer Sample Buffer to Intermediate Tubes**

- a) Locate the 250 mL twist-top bottle of sample buffer supplied in Pack # 1. Install the twist cap.
- b) Transfer about 5 mL of buffer to a sterilized disposable 15 mL intermediate working tube.

### **3. Add sample buffer to microcentrifuge tubes**

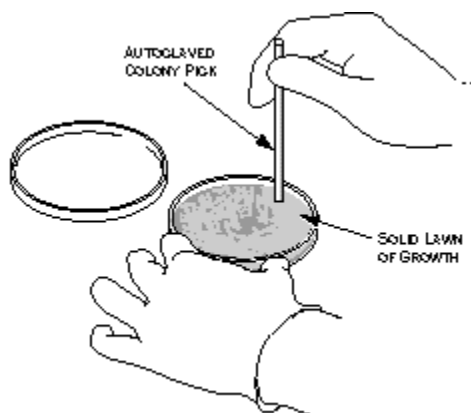
1. Place a sterile 0.65 mL microfuge tube in each of the eight holes in the lower row of the sample preparation rack.
2. For Gram negative samples (including *E. coli*), add 200 µL of sample buffer from the intermediate tube.

For Gram positive samples (e.g. *S. aureus* and *L. innocua* QC strains), add 40 µL of sample buffer.

3. Close the lids on the tubes.

#### 4. Harvest the Samples

1. Using autoclaved colony picks and making certain not to gouge the agar, carefully place the pick into one of the single colonies or the lawn. You need a sample area at least equal to that of the bottom of the colony pick. In most cases you will need to harvest from the lawn area of the plate. If you are working with large colonies, a single colony will be adequate.



2. For Gram negative samples (e.g. *E. coli*), perform 1 pick placed into 200  $\mu$ L of sample buffer.

CAUTION! Do not try to use the same pick twice on a plate. You need to harvest only enough sample to cover the bottom surface of the pick. Make sure the end of the pick is flat, if not, use a different pick.

CAUTION! Do not overload the harvesting pick. Collect only enough sample to cover the base of the pick. Over sampling will cause inaccurate results. Over sampling is a particular problem with *Staphylococcus*.

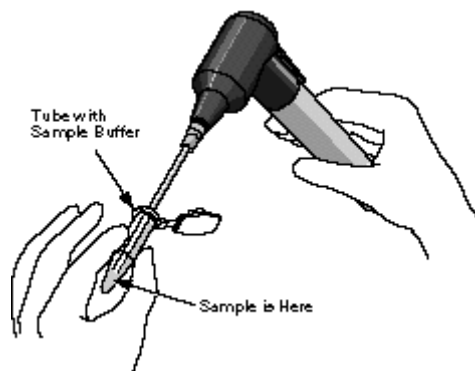
## 5. Mix the Samples

**WARNING!** Perform sample preparation using a Class 2 biological safety cabinet since aerosols may be formed during mixing of the samples.

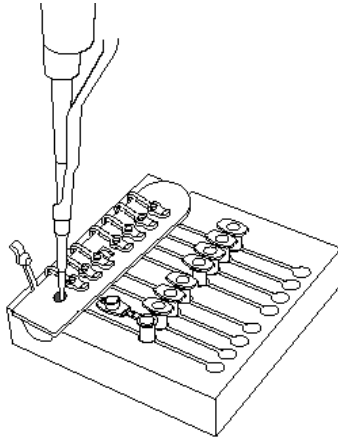
1. Making certain not to touch the sample end of the pick, place the pick into one of the filled sample tubes.
2. While holding the tube with the open end facing away from you, carefully attach the pick to the hand-held mixer. The fit of the pick in the coupling will be loose.

**WARNING!** Do not turn on the mixer unless the pick is inside the sample tube and below the surface of the liquid. Turning the unit on at other times will cause the sample to aerosolize and may cause contamination.

3. Press the ON lever on the mixer for about 5 seconds.
4. Release the lever and carefully remove the colony pick. The sample liquid should appear turbid.
5. For **Gram positive samples only**, (e.g. *Staphylococcus* and *Listeria*) locate a new colony pick and repeat the steps for harvesting and mixing samples, adding a second sample to the original tube. Discard the used picks in a biowaste bag.
6. Cap the sample tube.
7. Move the tube to the top row of the sample preparation rack. This indicates that the tube is filled.



## 6. Transfer the Samples to the Sample Carrier



1. Open the lid covering the first well of the sample carrier.
2. Using a 100  $\mu\text{L}$  pipetter, pipette 30  $\mu\text{L}$  of sample from the microcentrifuge tube into the well.
3. Close the lid cover for the well.
4. Repeat for remaining samples using a new pipet tip for each sample.

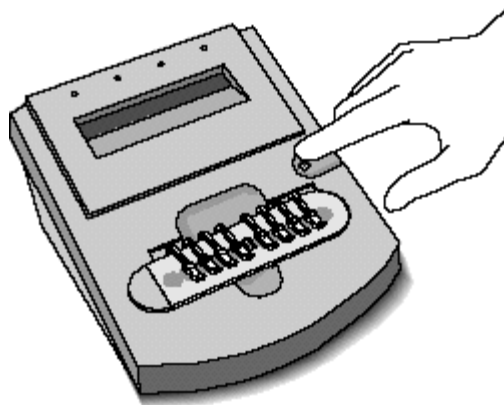
*CAUTION! Transfer the sample carrier to the Heat Treatment Station within 2 hours. If you wait longer than 2 hours, you will have to discard the sample carrier and begin again for this batch.*

6. Lightly wipe down the outer surfaces of the sample carrier with a lab wipe wetted with surface disinfectant (10% bleach or 70% alcohol).
7. Write down the name or code you use to identify the sample and the well number in the sample carrier for each sample using a sample log sheet.

## 7. Place the Sample Carrier in the Heat Treatment Station and Process the Sample Carrier

1. Place the sample carrier into the Heat Treatment Station. The display on the Heat Treatment Station will show **Insert**, if power is available. If the display is blank, make certain that the power cord on the back of the station is properly connected.

After you insert the carrier, the display shows **Press Button**.



2. Press the button on the Heat Treatment Station.

The display shows **Warm up** and counts down from **10** while the station is warming up. The actual warm up cycle varies with the condition of the room and the heat treatment station. Normal time is about 4 minutes.

When the station reaches operating temperature, the display changes to **Heat** and counts down from **13**. This represents each minute of heat treatment.

The indicator message changes to **Cool**. The display counts down from **9**, indicating the minutes remaining in the cooling cycle. If necessary, you can remove the carrier as soon as the **Cool** message appears.

3. The heat treatment step is finished when the display shows **READY** and counts down from **90**. The display will flash and an audible beep will sound three times. The alarm will then beep once every 10 minutes until the sample is removed or 90 minutes elapses.

*Caution! The heat-treated samples must be used within the 90-minute period at room temperature or they must be discarded. The heat-treated samples may be stored at this point (prior to adding Lysis Agents, if required) for 1 week at 4 °C, or for several months at -70 °C.*

## 8. Add the Lysing Agents (for *Staphylococcus* and lactic-acid bacteria only)

1. Using a 10- $\mu$ L pipetter and new tips for each addition, add 5  $\mu$ L of Lysing Agents A and B to each sample. Note: this step is omitted for *E. coli* as it has no effect on ribopatterns. Lysing Agents were specifically developed for *Staphylococcus* and Lactic-Acid bacteria samples.

*Caution! This step must be performed just prior (within 10 minutes) of loading the samples into the RiboPrinter and starting the run.*

### Creating and Loading a Batch

There are three options under the Operations menu for creating standard batches:

- [EcoRI batches \(VCA\)](#)
- [PstI batches \(VCB\)](#)
- [PvuII batches \(VCC\)](#)

You can also create special batches:

- Restriction Enzyme Flexibility batches
- **Substitute Enzyme batches (including *Hind III*)**

From the Instrument Control Base Window:

1. Move the pointer to Operations and click with the mouse button. The Operations menu appears.
2. Move the pointer to Create Substitute Enzyme Batch and click with the mouse button.
3. Use the View menu to remove any optional items you do not wish to fill in. The system requires at least Sample Type and RiboGroup Library information for each sample. You cannot remove these options. The **Clear** option de-selects the **Use Default ID Libraries**. You will have to enter a DuPont ID and Custom ID library name for all samples. These become required fields and the system will make you enter data before you can save the information in this window.

*CAUTION! If you change the display after you have entered information, you will lose all the information in the window. The window will redraw with a new blank display showing the items you have selected.*

4. To enter information about the sample, click on the **View** button with the mouse button, then click on **Sample Items**. Click on the options you want to display.
5. Enter your initials and any comment you want to record about the batch.
6. Select the lot number fields and record for all reagents.

*CAUTION! All fields must be completed or the system will not let you start processing the batch.*

7. For each well in the sample carrier, choose the type (Sample or Control [QC Number]) from the Sample Type field. The system defaults to Sample.
8. Once you define the Sample Type as Sample, type in the name you actually want to use. This information will appear as Sample Label in the Data Analysis software screens.
9. You can change the RiboGroup library name if needed. Do this by clicking on the button next to the field with the mouse button. A pop up menu appears listing your choices. If you want to add a new library name, move the pointer to the line and click with the mouse button to get a cursor, then type in the new library name. Once you have saved this file, the new name will be added to the pop up list for future use. Do **NOT** change the DuPont ID field. If you select one of the QC strains, the system automatically enters QC in the DuPont ID and RiboGroup Library fields. Do not change these names. If you wish, you may enter a name for the Custom ID library.
10. Repeat for the other seven samples.
11. Click on Save and Submit Batch to Instrument.

## Loading Disposables

Follow the screen prompts to load disposables and check the DNA Prep Waste. The icons on the window will flash red to tell you to remove and load an item. The screen prompts you about which Separation and Transfer chamber to use for the membrane and gel cassette. The LDD Pipette will move to physically block you from placing samples in the wrong chamber.

**CAUTION!** Do not try to move the pipette manually. You will cause the system to lose the step count. This can result in the loss of batch data. If the pipette is blocking the S/T chamber that you are instructed to use, STOP. [Call Customer Support](#).

**CAUTION!** Do not load disposables until you are prompted by the system. If you try to load them earlier, the alarm will sound as long as the doors are open. If you do load disposables ahead of time, the MP Base will be moved to the wrong position and you will not be able to begin processing the batch. You will not be able to move the MP base manually.

### 1. Check the DNA Preparation Waste Container

1. The DNA Prep waste container must be visually checked before every batch. If the container looks nearly full (about 1 inch from the top), remove the container, unscrew the cap and empty into the liquid biohazard waste.

**WARNING!** Do not tip the DNA Preparation waste container when you remove it.

**WARNING!** Do not unscrew the cap from the DNA Preparation waste container if the fluid level has risen into the cap. First pour the excess waste liquid into the liquid biohazard waste.

**WARNING!** When replacing container make sure that the cap is properly threaded in place. If the cap is only partially threaded, it can snag the pipette during operation.

### 2. Load the Sample Carrier

1. Place the sealed carrier into the labeled slot on the far right of the characterization unit.
2. Push the sample carrier down firmly until it snaps into place.

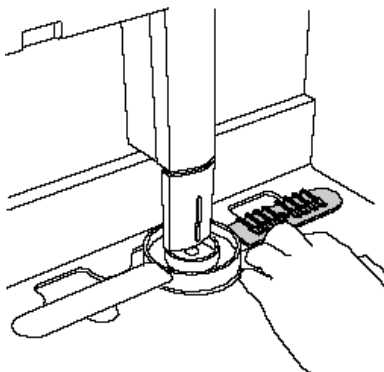
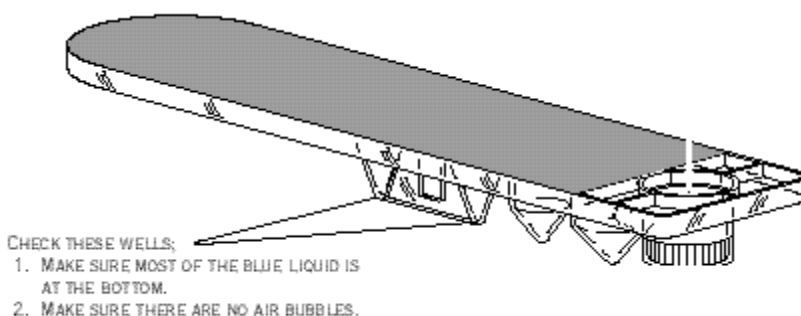
**CAUTION!** Place the rounded edge of the sample carrier on your right as you view the characterization unit. Position the carrier this way to insure correct identification of the sample wells.

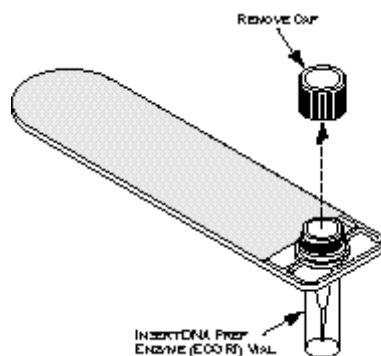
### 3. Load the DNA Prep Carrier

1. Remove the DNA Prep carrier from the refrigerator.
2. Check the wells in the carrier. If most of the liquid appears to be in the bottom of the wells and there are no bubbles, go to step 3. Otherwise **lightly tap the side of the carrier a few times with your finger to release any material that has adhered to the lid.**
3. CAUTION! Do not tap the carrier briskly. This may cause the marker to degrade which can create inaccurate results.
4. Remove a vial of DNA Prep Enzyme (*Hind* III or *Eco*R I) from the freezer. ***Hind* III (NEB Cat. #R0104M) is prepared in a Sarstedt 500- $\mu$ L microfuge tube (Cat. #72730-005) as a 50 U/ $\mu$ L working stock as follows.**

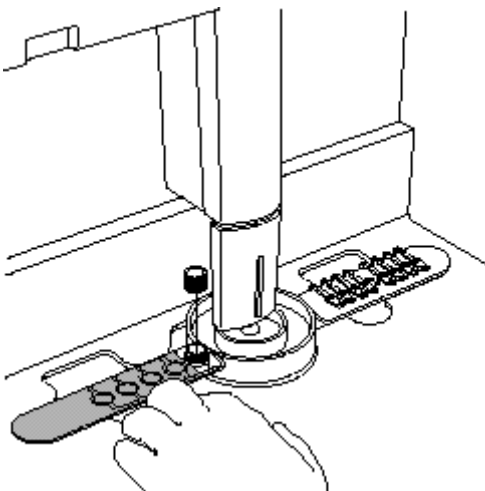
50 U/ $\mu$ L: 26.5  $\mu$ L *Hind* III and 26.5  $\mu$ L of NEB 10X Buffer 2

**During addition of the Buffer, mix enzyme and buffer to homogeneity by stirring with the micropipette tip.**



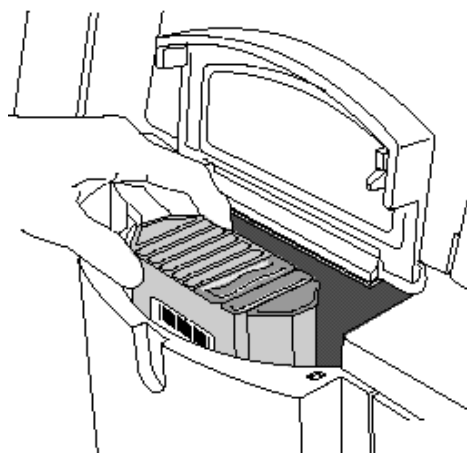
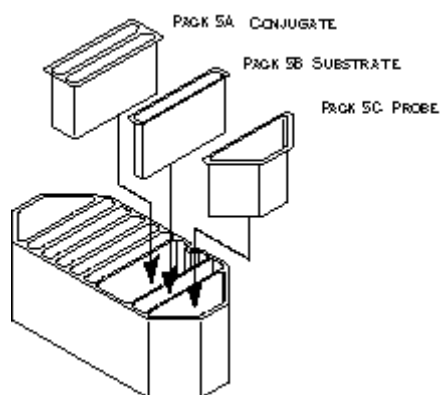


5. Remove the cap from the Enzyme vial.
6. Insert the vial into the carrier.
7. Place the DNA Prep carrier into the slot labeled **Reagent** to the left of the sample carrier slot.
8. Push the DNA Prep carrier down firmly until it snaps into place.



#### 4. Load the MP Base and Carousel

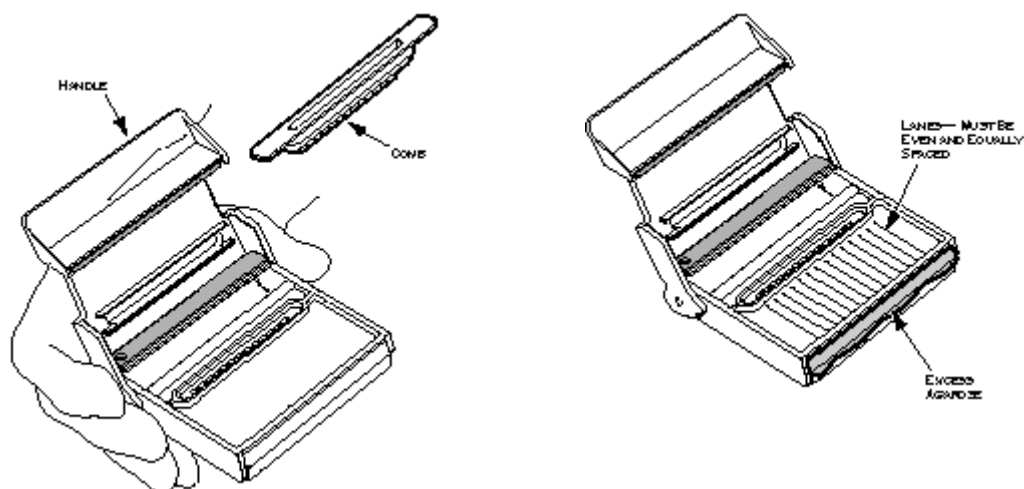
1. Unpack the disposables.
2. Remove the MP base (Pack 5) from the incubator and the Conjugate (Pack 5A), Substrate (Pack 5B), and Probe (Pack 5C) from the refrigerator.
3. Remove each insert from its pouch. Tap the powdered reagent packs gently to bring all powder to the bottom of the packs. Place reagent packs in the MP base and load the base in the carousel.



**CAUTION!** Push each insert firmly into place. If part of the insert extends above the top of the base, it could catch on the bottom of the deck and cause a system error. You could lose one or more batches as a result. Each insert is keyed by shape and cannot be inserted incorrectly.

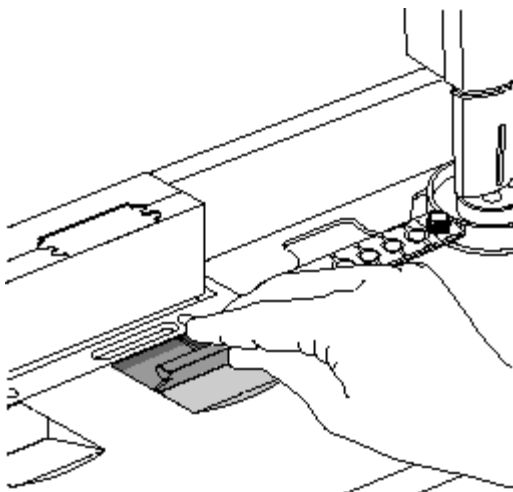
## 5. Load the Gel Cassette

1. Remove the gel cassette from its package.
2. Grasp one end of the rubber comb and gently pull the comb from the cassette.
3. Unfold the handle of the cassette towards you until the handle snaps into place.
4. Check the front edge of the gel cassette and the lanes of the gel.



**Warning!** If the cassette shows a build up of excess gel on the front edge, or if you notice any shrinkage of the gel away from the cassette or bubbles, record the lot number and call Customer Support. Use a new cassette for this run.

5. Insert the gel cassette into the slot labeled **Gel Bay**. The RiboPrinter® system will prevent the insertion of the cassette into the incorrect slot by blocking one slot with the LDD Pipette.

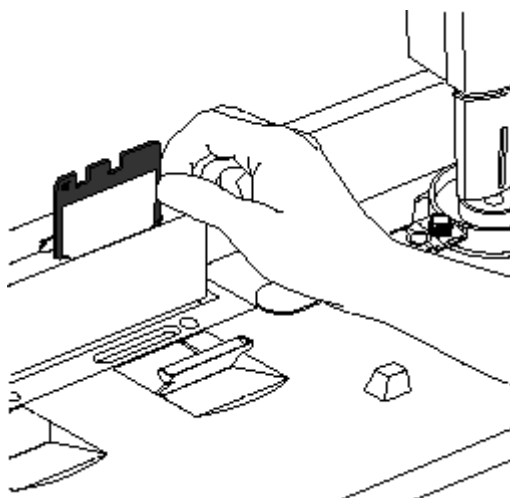


6. Press the cassette forward firmly until it snaps into place.

## 6. Load the Membrane

1. Grasp the membrane and carefully drop it into the front slot and flip the metal bracket against the back of the membrane.

*CAUTION! You can insert the membrane backwards. This will cause an alarm that prevents the sample from being processed until the error is corrected. Always make certain that the two large slots are on top and that the square hole on the side faces your left as you insert the membrane.*



**7. Close all doors and the instrument will begin sample processing.**

## 8. Load the Next Batch

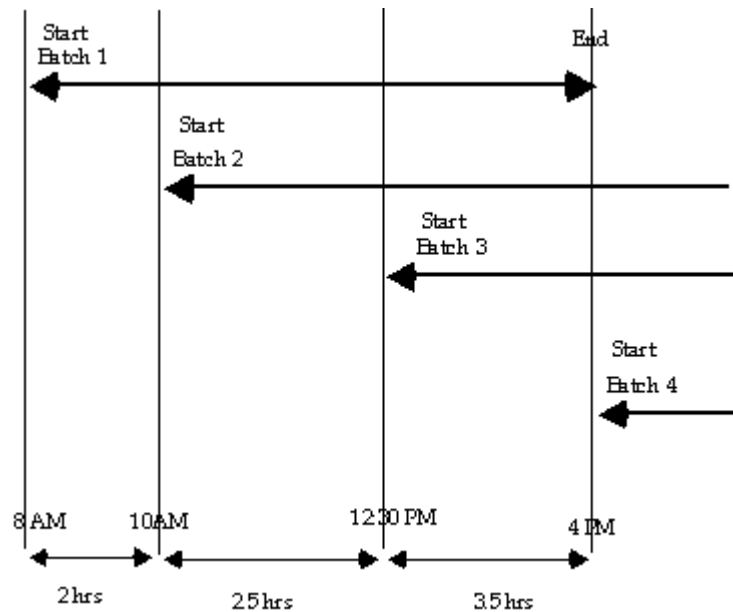
The RiboPrinter® microbial characterization system lets you load up to four VCA batches in an eight hour period. Other batches may take longer to process.

The chart above shows the approximate loading times for each batch in a work shift using only the VCA protocol.

1. You can now use the **Create Batch** option to set up a new pending batch.
2. When you complete the information window and click on the **Start Normal Batch** option, the window displays a message telling you when you can load the next batch.

## Batch Report

After image processing is completed, the system automatically runs a series of analysis functions and generates a Batch Information Report. This task does not require any action on the part of the operator. Reports are automatically saved to the hard disk of the computer and sent to the printer.



Casarez, E. A.; Pillai, S. D.; Mott, J.; Vargas, M.; Dean, K.; Di Giovanni, G. D. (2007). "Direct comparison of four bacterial source tracking methods and a novel use of composite data sets." J. Appl. Microbiol. In press doi:10.1111/j.1365-2672.2006.03246.x.

## **B-4: *Bacteroidales* PCR**

### **Preprocessing of Water Samples**

1. Within six hours of sample collection, water samples (100 ml) are filtered through 0.2  $\mu$ m pore size Supor-200 filters (VWR cat # 28147-979). Discard filtrate and place the filter into a pre-labeled 7 oz Whirl-Pak bag using ethanol-flamed forceps and aseptic technique. If 100 ml of water cannot be filtered, record the volume filtered on the Whirl-Pak bag and COC.
2. Add 500  $\mu$ l of guanidine isothiocyanate (GITC) lysis buffer to each Whirl-Pak bag with filter.  
  

100 ml of GITC lysis buffer  
50 ml reagent grade (deionized) water  
59.08 g GITC (VWR # 100514-046; 5 M final)  
3.7 g EDTA [pH 8.0] (VWR # VW1474-01; 100 mM final)  
0.5 g Sarkosyl (VWR # 200026-724; 0.5% final)  
Adjust to pH 8.0 with NaOH (approx. 0.4 g of pellets) to dissolve EDTA and heat with vigorous stirring to dissolve guanidine  
Bring up to 100 ml total volume with reagent grade (deionized) water  
Autoclave and store at room temp
3. Store samples at -80°C (or -20°C manual defrost freezer, not the standard auto-defrost).
4. After processing, samples should be shipped overnight on dry-ice to SAML. Dry-ice blocks should be packed on both top and bottom of the cooler for shipment. Extra care should be taken to ensure filters do not thaw in transport by not overcrowding the cooler and using adequate amounts of dry ice.
5. Notification of shipment should be sent to SAML via email, [emartin@ag.tamu.edu](mailto:emartin@ag.tamu.edu), or phone, SAML Lab 979-845-5604, no later than the day of shipping. Notification should include tracking number and direct Ana-Lab contact person for confirmation upon receipt of samples.
6. DNA will be extracted from the samples and analyzed by *Bacteroidales* PCR as described below.

### **DNA Extraction and PCR**

1. DNA is extracted from the water concentrates using QIAamp DNA mini kit. Turn on the slide warmer and set to maximum. Preheat a microfuge tube rack and 0.01X TE buffer pH 8.0 for elution and a 70°C water bath.
2. Add 500 µl of Buffer AL to each thawed tube and vigorously agitate for 1 min using a wrist action shaker.
3. Incubate in a 70°C water bath for 10 minutes.
4. Transfer lysate to a 2.0 ml microfuge tube.
5. Add 500 µl of 100% ethanol and pulse vortex mix for 15 sec. Quick spin to remove droplets from cap.
6. Transfer half of the sample lysate (600 to 750 µl) to a labeled QIAamp column placed in a Qiagen collection tube. Microfuge at 14K rpm, with brake, for 1 minute. If necessary, at each step wipe off any buffer from outside of column with a lab tissue before placing into a new collection tube.
7. Place column in a new collection tube and repeat Step 6 with the remaining sample.
8. Place column in new collection tube and add 500 µl of AW1 wash buffer. Centrifuge as above and place column in a new collection tube.
9. Add 500 µl of AW2 wash buffer and centrifuge as above, then repeat once more. Place column in a clean collection tube and centrifuge as above to remove all traces of AW2 buffer.
10. Place in a clean collection tube in the heated rack on the slide warmer. Add 100 µl of 70 to 80 °C 0.01X TE buffer pH 8.0 and let incubate at 70 to 80 °C for 5 minutes with columns capped.
11. Immediately centrifuge at 14K rpm for 3 minutes and transfer the filtrate containing the eluted DNA to a labeled 0.65 ml tube. Store at -80 °C until analyzed by PCR. Keep the remainder of the unused aliquot of 0.01X TE to use as a no template control for the PCR.

***Bacteroidales* PCR Master Mix**

1. Prepare sufficient PCR Master Mix for samples and controls, as well as one blank per 10 samples to account for volume loss due to repeat pipetting.

***Bacteroidales* PCR Master Mix – per sample**

<b>MASTER MIX</b>	<b>Amt (uL)</b>	<b>Final Calc</b>	<b>Final Units</b>
Molecular Grade Water	<b>30.2</b>		
10X PCR buffer I w Mg (ABI)	<b>5</b>	1	X
MgCl <sub>2</sub> (25 mM) (ABI)	<b>1</b>	0.5 (2.0 final)	mM
each dGTP, dCTP, dATP (33 mM mix) (Amersham)	<b>0.3</b>	200	uM each
dUTP (100 mM) (Amersham)	<b>0.2</b>	400	uM
Bacteroidales Primer Mix	<b>5</b>	200	nM each
BSA (30 mg/mL)	<b>2.5</b>	1.5	ug/uL
AmpliTaqGold (Units)	<b>0.5</b>	2.5	Units/rxn
Uracil DNA glycosylase NEB (UDG; 1 U/rxn)	<b>0.25</b>	0.5	Units/rxn

2. Dispense 45 µl of Master Mix for each sample into the appropriate well of PCR plate.
3. Briefly vortex DNA extracts, quick spin, then add 5 µl to the appropriate PCR well.
4. Carefully seal plate using an adhesive PCR cover.
5. Load the plate into the thermal cycler and run under the appropriate *Bacteroidales* program with the following cycling conditions:
  - a. UDG digestion 50°C for 10 min
  - b. Initial denaturation at 95°C for 10 min
  - c. 40 Cycles:
    - i. Denaturation at 95°C for 30 sec
    - ii. Annealing at 53°C to 62°C (depending on primer set) for 1 min
    - iii. Extension at 72°C for 1 min
  - d. Final Extension at 72°C for 10 min
6. Store completed reactions at -20°C until analyzed by gel electrophoresis.
7. Prepare a 200 mL, 2% agarose gel using a 500 mL bottle. Add 200 mL of 1 X TBE buffer and 4.0 g agarose. Microwave until agarose is fully dissolved, add 10 µl of ethidium bromide (10 mg/ml), tighten cap, swirl to mix and let cool 1-2 minutes.

8. Pour agarose into casting tray with one or two 20-tooth, 0.75 mm thick combs.
9. Allow gel to solidify for 30-60 minutes on the bench, remove comb(s), and place in gel tank with TBE buffer. Discard TBE in gel tank after it has been used twice.
10. The following items will be needed for electrophoresis:

100 bp ladder (0.33 µg/10 µL) (1500 µL final, enough for 150 lanes)

200 µL Roche DNA Marker XIV (Cat. #1721933) 0.25 µg/µL 100 bp ladder (add reagents below to a full tube of marker)

300 µL 6X Loading Buffer (see recipe below)

150 µL 10X PCR buffer

850 µL molecular grade water

Store in cold room

6X Loading Buffer

25 mg bromphenol blue (0.25%)

1.5 g ficoll 400 (15%)

Add molecular grade water to 10 mL, divide into 1 mL aliquots and freeze, the aliquot currently being used can be stored in the cold room

11. Mix 10 µl of PCR product with 2 µl of 6X Loading Buffer in the appropriate well of a Nunc Module.
12. Load the gel, starting with 10 µl of 100 bp ladder in the first lane, followed by 12 µl of each sample with Loading Buffer, and 10 µl of 100 bp ladder after the last sample.
13. Start electrophoresis power supply set at 100 volts, run for 1.5 hours.
14. Follow Gel Imager SOP for image capture. Save digital photograph as an 8-bit TIFF file with no scaling and print a hardcopy for notebook.

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## **APPENDIX C. Sample Handling and Shipping for EPA Method 1603**

### **Shipping and Handling of modified mTEC plates from EPA Method 1603**

1. After 22 +/- 2 hour incubation, red or magenta colonies are considered 'typical' *E. coli*.
2. Colonies counted should be indicated with a 'dot' on the back of the plate to ensure isolation of *E. coli* grown during the incubation period. Total number of counts should also be included on the back of each plate. In order to facilitate isolations, include at least one plate per sample having a countable number of *E. coli* colonies (20-80/plate).
3. Each plate should be sealed with parafilm around the edge to protect the filters from contamination. Dilution series for each sample should subsequently be grouped together either by parafilm or zip-top bag for transport.
4. The day following filtration, but no later than two days following filtration, plates should be shipped overnight to SAML at 4°C. 'Blue-ice' or freezer blocks should be used to keep the samples cool, but not frozen in transport. Samples should be placed in secondary containment such as large Whirl-Pak or zip-top bags.
5. If sampling occurs over two days, the first day's plates should be counted 24 hours post filtration, sealed and placed 'media-side up' or 'upside down', so condensation does not fall onto the filter, and stored 4°C until a complete sample set can be shipped together the next day.
6. Notification of shipment should be sent to SAML via email, [emartin@ag.tamu.edu](mailto:emartin@ag.tamu.edu), or phone, SAML Lab 979-845-5604, no later than the day of overnight shipping. Notification should include *E. coli* count datasheet, tracking number, and direct Ana-Lab contact person for confirmation upon receipt of samples.

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## **APPENDIX D. CHAIN OF CUSTODY FORM**



Ana-Lab Corp. P.O. Box 9000 Kilgore, TX 75663

Phone 903/984-0551 FAX 903/984-5914 e-Mail corp@ana-lab.com

LELAP-accredited #02008

07/23/2009 Page 1 of 1

## Chain of Custody

### Report To

Randy Rushin  
Water Monitoring Solutions  
PO Box 1132  
Sulfur Springs, TX 75483-

WMS2

103

*E. coli by SM9223 B.a.*

Lab Number

Phone 903/439-4741

Fax 903/439-0021

Accredited Test Name Method

Matrix: Liquid Aqueous  
Sample Collection Start

Date: Time:

Sampler Printed Name:

Sampler Affiliation:

Sampler Signature

\*\*Na2S2O3 (0.006%) Polystyrene-100 mL Sterilized

N \* MPNE MPN, E coli (Coliform-18) SM 9223 B.a, 20th Ed

### Ambient Conditions/Comments

Date	Time	Relinquished		Received	
		Printed Name	Affiliation	Printed Name	Affiliation
		Signature		Signature	
		Printed Name	Affiliation	Printed Name	Affiliation
		Signature		Signature	
		Printed Name	Affiliation	Printed Name	Affiliation
		Signature		Signature	
		Printed Name	Affiliation	Printed Name	Affiliation
		Signature		Signature	

Sample Received on Ice? ☐ Yes ☐ No Method of Shipment: ☐ UPS ☐ Bus ☐ FedEx ☐ Lone Star ☐ Hand Delivered ☐ Other

Cooler/Sample Secure? ☐ Yes ☐ No Tracking/Shipping #

The accredited column designates accreditation by A - A2LA, N - NELAP, or S - not covered under A2LA or NELAP scope of accreditation. Ana-Lab personnel collect samples as specified by Ana-Lab SOP #000323.

Comments:

Corporate Shipping: 2000 Dudley Rd. Kilgore, TX 75663



ISO-17025 # 0637-01

LISClient v1.0.1.306



NELAP-accredited #T104704201-08-TX

www.ana-lab.com



2008 Seal of Excellence

Form rptccol Created 06/22/2004 v1.3



Ana-Lab Corp. P.O. Box 9000 Kilgore, TX 75663

Phone 903/984-0551 FAX 903/984-5914 e-Mail corp@ana-lab.com

LELAP-accredited #02008

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## Chain of Custody

### Report To

Randy Rushin  
Water Monitoring Solutions  
PO Box 1132  
Sulfur Springs, TX 75483-

WMS2

104

BSI Prep (EPA 1603)

Lab Number

Phone 903/439-4741

Fax 903/439-0021

Accredited Test Name Method

Matrix: Liquid Aqueous

Sample Collection Start

Date: \_\_\_\_\_ Time: \_\_\_\_\_

Sampler Printed Name: \_\_\_\_\_

Sampler Affiliation: \_\_\_\_\_

Sampler Signature: \_\_\_\_\_

AN

\*\*Na2S2O3 (0.008%) Polystyrene-100 mL Sterilized

• 1603

E-coli (mTEC mod)

EPA 1603

Z1-Administrative use only: no bottle required

SHIP

Shipped Overnight

### Ambient Conditions/Comments

Date	Time	Relinquished	Received
		Printed Name _____ Affiliation _____	Printed Name _____ Affiliation _____
		Signature _____	Signature _____
		Printed Name _____ Affiliation _____	Printed Name _____ Affiliation _____
		Signature _____	Signature _____
		Printed Name _____ Affiliation _____	Printed Name _____ Affiliation _____
		Signature _____	Signature _____
		Printed Name _____ Affiliation _____	Printed Name _____ Affiliation _____
		Signature _____	Signature _____

Sample Received on Ice? ☐ Yes ☐ No

Cooler/Sample Secure? ☐ Yes ☐ No

Method of Shipment:

☐ UPS

☐ Bus

☐ FedEx

☐ Long Star

☐ Hand Delivered

☐ Other

Tracking/Shipping #

The accredited column designates accreditation by A - A2LA, N - NELAP, or Z - not covered under A2LA or NELAP scope of accreditation. Ana-Lab personnel collect samples as specified by Ana-Lab SOP #000323.

Comment:

Corporate Shipping: 2800 Dudley Rd. Kilgore, TX 75662



ISO-17025 # 0637-01

LDSC Item v1.0 1.306



NELAP-accredited #T104704201-08-TX

www.ana-lab.com



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Form rptccol Created 06/22/2004 v1.5



Ana-Lab Corp. P.O. Box 9000 Kilgore, TX 75663

Phone 903/984-0551 FAX 903/984-5914 e-Mail corp@ana-lab.com

LELAP-accredited #02008

## Chain of Custody

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### Report To

Randy Rushin  
Water Monitoring Solutions  
PO Box 1132  
Sulfur Springs, TX 75483-

WMS2

105

Bacteroidales Prep

Lab Number

Phone 903/439-4741

Fax 903/439-0021

Accredited Test Name Method

Matrix: Liquid Aqueous  
Sample Collection Start

Date: Time:

Sampler Printed Name:

Sampler Affiliation:

Sampler Signature:

AN	**Na2S2O3 (0.005%) Polystyrene-100 mL Sterilized
BPMF	Bacteroidales Prep - MF
Z1-Administrative use only: no bottle required	
SHIP	Shipped Overnight

### Ambient Conditions/Comments

Date	Time	Relinquished	Received
		Printed Name Affiliation	Printed Name Affiliation
		Signature	Signature
		Printed Name Affiliation	Printed Name Affiliation
		Signature	Signature
		Printed Name Affiliation	Printed Name Affiliation
		Signature	Signature
		Printed Name Affiliation	Printed Name Affiliation
		Signature	Signature

Sample Received on Ice? ☐ Yes ☐ No Method of Shipment: ☐ UPS ☐ Bus ☐ FedEx ☐ Lone Star ☐ Hand Delivered ☐ Other  
Cooler/Sample Secure? ☐ Yes ☐ No Tracking/Shipping #

The accredited column designates accreditation by A - A2LA, N - NELAP, or 2 - not covered under A2LA or NELAP scope of accreditation. Ana-Lab personnel collect samples as specified by Ana-Lab SOP #000323.

Comments:

Corporate Shipping: 1600 Dudley Rd. Kilgore, TX 75662



ISO-17025 # 0637-01



NELAP-accredited #T104704201-08-TX



2008 Seal of Excellence

LDSClient v1.0.1.306

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